MEDICAL PROCEEDINGS

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IN THIS ISSUE · IN HIERDIE UITGAWE

Studies on the Repair of Cutaneous Wounds

I. Healing of Incised Wounds, with Reference to Epidermal Reactions to Sutures, and the Pathogenesis of Carcinoma in Scars

II. The Healing of Wounds Involving Loss of the Superficial Portions of the Skin

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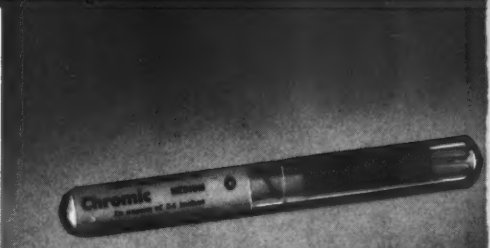

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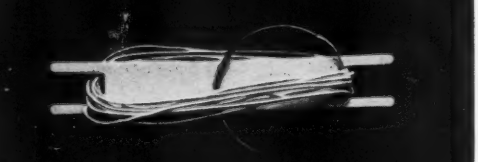

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STUDIES ON THE REPAIR OF CUTANEOUS WOUNDS*

I. HEALING OF INCISED WOUNDS, WITH REFERENCE TO EPIDERMAL REACTIONS TO SUTURES, AND THE PATHOGENESIS OF CARCINOMA IN SCARS

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SUMMARY

The healing of incised wounds of the skin and the reactions to sutures used in closing them has been re-examined in serial sections of biopsy material taken from human volunteers, from rats and from rabbits, dating from the first day to many years post-operatively. Contrary to generally held opinions, the first signs of repair are detectable in the epidermis and not in the connective tissues. The regenerating epithelium bridges the wound first. Connective tissue regeneration, in the dermis in particular, *follows* epidermal coverage of the incision; transected muscle and fat may show fibrotic changes earlier than does the dermis.

The epidermis at the wound edges always becomes inverted and then grows into the incision, making the healing wound initially V-shaped. Surface convexity of scars only develops after several weeks, when considerable amounts of fibrous tissue have been formed in the dermis. Once the regenerating epidermis has bridged the incision, it tends to thicken rapidly and to invade the underlying connective tissue in spurs which, in turn, appear to play a part in evoking the regeneration of new connective tissue, particularly in the incised stratum papillaris of the dermis. The invading epithelial spurs are subsequently, in the main, treated by the connective tissues as foreign bodies, with consequent localized epithelial pearl-formation deep in the healing incision. New hairs and new glands, especially sebaceous glands, may also develop within the invading epidermal spurs. These epidermal reactions may be as marked at the suture

* This contribution is dedicated to Mr. John S. Schlesinger, in appreciation of his interest in the problems and progress of medical research in South Africa.

needle puncture wounds and along the suture tracks, as at the incision itself.

The genesis of suture needle puncture wound scars is described. Epidermis is shown to invade very deeply into dermis, along sutures. The adverse effects on healing, and on the residual scar, of the epidermal reactions to sutures are noted. In the light of these findings, it is suggested that new, sutureless methods for wound closure be sought.

Initial dermal changes are alterations in staining of collagen. True fibroblastic proliferation in the cut stratum reticularis of the dermis seems to follow and, in part, to be dependent upon the epidermal reactions at the surface and on the fibrosis in deeper-lying transected subcutaneous fat and muscle. The tensile strength of the incision, during the first 3-5 days, seems to be a function primarily of regenerated epithelium and connective tissue repair in fat, in muscle and around sutures. The last layer to heal by fibrosis is the transected stratum reticularis of the dermis. The epidermal invasions related to healing wounds and the associated connective tissue changes are shown to persist for weeks, if not for months.

Attention is drawn to the initiation of healing by the epidermis, and the subsequent regulation of epidermal invasiveness by the ability of the connective tissues to respond to the epidermal evocator. Neogenesis of hair follicles and associated glands is another of the results of epidermal-dermal inter-reactions during healing.

Similarities are shown to exist between the epidermal and dermal reactions in wound healing and those in the early stages of carcinogenesis. The genesis of carcinomata in scars is discussed in the light of this observation.

INTRODUCTORY REVIEW AND REMARKS ON THE REPAIR OF CUTANEOUS WOUNDS

The classic studies by Loeb and his associates, and by other workers who investigated various aspects of wound healing, have been fully reviewed by Arey[2] and also by Needham.[68] These studies dealt primarily with the nature of healing processes, with particular emphasis on the manner and rate of tissue reorganization in the site of injury and the factors which might expedite this repair process. Reports by Ragan *et al.*[80, 81] concerning the effects of cortisone on wound healing have provoked world-wide interest in the effects of steroid hormones on the healing process, on inflammation and on responses to grafts. Much of this literature has been surveyed.[3-5, 13, 89-91]

Other practical problems arising mainly from the expanding activities of modern surgeons, such as the merits of various types of suture material, have also received close attention. The outstanding studies and reviews by Localio *et al.*[52, 53] are an extremely useful guide to the relevant techniques and literature. The importance of wound size and depth and of the infliction of repeated injury in modifying the rate of healing processes, has been carefully investigated,[15, 35, 42, 43, 48, 82, 83] while the implications of such studies for an understanding of the possible rôles of 'wound hormones' in the healing process have also been reviewed.[16, 17]

Recently we drew attention to several reactions which supervene in spontaneously healing *excised* wounds.[27] During the course of the above study, many aspects of reactions in wounds, in grafts of different types and in the graft host sites were examined and described.

Although we accepted the prevailing view at that time, we were *not* convinced that the fibrous tissue, which we constantly saw uniting grafts to the host site, had in fact developed within the blood, shed at the time of operation, or even within the plasma which is alleged to ooze from damaged blood and/or lymphatic vessels at the site of injury. This latter is a generally recorded and accepted view concerning the regeneration of connective tissue within a wound.[1, 10, 21] At the time of writing our previous report, we tacitly accepted this view and interpreted our data accordingly. However, we had already made several observations which caused us to suspect the validity of this view and our further reading of the earlier literature intensified our uncertainty.

For the plastic surgeon, the accumulation of blood below a graft constitutes a recognized cause of graft failure; meticulous care is taken to avoid such a complication. Yet, to most pathologists, the presence of blood, clotted plasma or at least lymph, is regarded as a basic essential in the repair process. The exuded blood, plasma or lymph are considered to act by 'gluing' the 2 sides of the wound together and by providing both the pabulum and supporting framework promoting the growth into a wound of fibroblasts, vascular buds and all the other cells and structures involved in the repair process. Thus, Boyd[11] states: 'The coagulated plasma between the edges of the wound plays the part of a

medium, into which the fibroblasts grow and establish connection with those on the other side.' Paradoxically, the author of this standard text remarks, a little earlier in the same edition, that primary union of an incised wound' is attained with greater certainty if there is good apposition of the cut edges and when 'there is hardly any exudate between the surfaces'. The generally accepted view, and one in which both pathologists and surgeons concur, is that the presence of a blood clot in the wound delays the healing process and may prevent primary union in an incised wound.

No one who has examined, even macroscopically, an incised wound within 5 to 10 minutes after the cut surfaces have been apposed, will doubt that a fibrin-like clot glues these edges together. But the rôle of this clot in the subsequent healing process, and the fate of the shed blood, is, in the light of our more recent findings, not as clear as is generally stated; nor is there unanimity about the origin of the new connective tissue which bridges the wound. Most textbooks assert that fibroblastic regeneration is initiated by the activation of fibrocytes lying dormant among the closely packed collagen fibres of the stratum reticularis of the dermis. Bishop [10] attributed considerable importance to the loose, cellular sub-epidermal and perifollicular connective tissue, while Hartwell [38-41] averred that the adipose tissue, with associated haematogenous 'macrophages', gave rise to the new fibrous tissue during wound dealing.

We found that during the repair of an incised wound the first signs of fibroblastic proliferation were regularly encountered within traumatized fat and muscle and, in the case of the dermis, *not* within or immediately adjacent to the incision, but rather some distance *away from* the incision (approximately 2-3 mm. away from the injury) in the neighbouring, apparently undamaged, slightly congested and oedematous stratum reticularis. Much later a *new* exudate appeared within the dermal part of the incision, and only then were fibroblasts and vascular buds found invading the line of incision itself; even then, these usually extended from the fat upwards and from the subepithelial zone downwards into the inert stratum reticularis.

Another generally accepted view is that an *incised* wound heals by the formation and subsequent organization of 'granulation tissue' of the type usually encountered in healing *excised* wounds (i.e. where some tissue has been lost during trauma or operation). Our findings are, once more, *not* in conformity with this view; nor could we find any close similarity between the granulations which develop in an *excised* wound and the connective tissue reactions during the healing of an *incised* wound. It is true that in both these instances the repair tissue is comprised of new vascular buds, fibroblasts, reticulin and other associated fibres and cells. However, the pattern of organization and the sequence of events in the two types of healing processes are quite different, in the same way as the architecture of the spleen, of lymph nodes, areolar connective tissue and tendon, differ profoundly even although they are all constituted of the same basic elements.

In our previous report we recorded some of the changes which supervene in 'granulation tissue' developing within an 'excised' wound or burn, once an epithelium (derived from the edges of a wound or from a graft) covers the surface of such granulations. Since that time we have conducted further experiments, both in animals and in human volunteers, which have allowed us to define, with greater precision, the course of events during healing. The apparent contradictions between views expressed in the literature and our own observations, and the absence from the available literature of reference to many striking phenomena which we encountered regularly in our material, prompted us to extend our studies of the repair process in different types of wounds.

In the present report attention will be devoted primarily to an analysis of some of our findings during the repair, by primary union, of *incised wounds* inflicted for experimental reasons on human and animal subjects. Subsequent reports in this series will deal with other aspects of repair in different types of wounds under a variety of experimental conditions.

MATERIAL AND METHODS

This study is based on material derived from both human and animal sources.

The human material is represented predominantly by a series of biopsies taken on a single healthy subject from 2 separate incisions each 6 inches long, made for the specific purpose of this investigation. The first of these wounds was inflicted on the anterior aspect of the thigh and the second operation was performed 8 months later on the abdomen. These incisions were made under aseptic conditions. The first incision was closed by

interrupted 5-0 silk sutures, and most careful attention was devoted to ensure accurate apposition of the cut surface. Elliptical biopsies were taken across this first incised wound on the thigh at 24 hours, 2 days, 4 days, 8 days and 10 days after the wound had been made. The operation and the biopsies were all performed under hypnotic analgesia and no local anaesthetic was used. The wound was dressed with *tulle gras* gauze which was covered by lint and bandaged.

The second wound, made across the subumbilical portion of the abdomen, was closed by interrupted sutures of different materials (silk, catgut, wire, nylon) and biopsies were taken at 4, 6, 8, 14 and 16 days post-operatively.

Further human material was obtained at operation from a number of human subjects with various types of scars resulting from incised-type wounds (Table I).

Animal Material: (*a*) *Rabbits*. The experimental procedure was as follows:

Rabbits weighing 1,500 to 3,000 g. were used. The hair of the lateral aspects of the thorax and abdomen of both sides was clipped short with electric clippers the day before operation. On the day of operation the animals were starved and ½ hour before operation they were injected subcutaneously with 1/150 grain of atropine. In the earlier experiments anaesthesia was induced with intravenous sodium pentothal and maintained with open ether. In later experiments intravenous nembutal (Abbott's 'Veterinary Nembutal') was employed. After the animals were anaesthetized, the hair on both sides of the thorax and abdomen was shaved with a sharp razor blade, special care being taken to avoid inflicting accidental small incisions or scraping the skin unduly. The area thus bared, extending from the level of the scapula to the iliac crest, was thoroughly cleansed with 2% 'Cetavlon' and dressed temporarily with cotton wool soaked in this antiseptic. All operations were carried out under sterile conditions, the efficacy of our precautions in this regard being evidenced by the fact that, in all operations performed, sepsis supervened in only 2 animals.

An incision 4 inches long was made with a sharp scalpel down through the panniculus carnosus to subcutaneous fat, care being taken to ensure that the wound was of the same depth throughout its length. Haemostasis was usually achieved by pressure with a dry gauze swab, and only on isolated occasions was it necessary to clamp a bleeding vessel and tie it with 4-0 catgut. The wound was closed with one layer of interrupted sutures placed at approximately ½-inch intervals. Number 4-0 black silk and a number 16 half-curved cutting suture needle were used. Care was taken to see that the sutures were not drawn too tight and that cut edges were carefully apposed. No antiseptics were applied to the wound at any stage of the operation or post-operative treatment. The wound was dressed with sterile, vaseline-impregnated gauze which in turn was covered with sterile dry plain lint. The animal was then turned on to its opposite side, the dressing on the operated side now being in contact with a sterile towel. After following the procedures outlined above, on the second side, the whole operated area was encased in a plaster of Paris bandage in the way recommended.[8] The animals were then returned to their pens and allowed free access

TABLE I: SUMMARY OF HUMAN BIOPSY MATERIAL OF INCISED WOUNDS

Type of Tissue		*Number of Specimens*
Uncomplicated Incised Wounds—Biopsies		
Post-operative Day:	1	3
	2	3
	4	4
	6	3
	8	5
	10	3
	14	3
	16	3
	17	3
Keloid Scars		9
Other Scars		28

TABLE II: SUMMARY OF BIOPSY MATERIAL OF INCISED WOUNDS OBTAINED FROM EXPERIMENTAL ANIMALS

Post-Operative Day ..	3—5	6—7	8—9	10—12	14—17	22—24	28—32	40—65	*Total Number of* *Animals*	*Biopsies*
Rabbit	6	5	2	8	4	1	2	2	9	30
Rat	12	11	1	4	8	1	7	6	35	50

to food and water. The schedule of times and the number of biopsies performed on each animal is outlined in Table II.

All biopsies were performed under the anaesthetic and working conditions outlined. After anaesthesia of the animals the plaster bandage was removed by cutting with plaster shears, care being taken not to crush or traumatize the skin in any way during this procedure. Moreover, on removing the plaster, precautions were taken to disturb the wound as little as possible. All biopsies were elliptical in shape and made across the line of the initial incision. In view of the findings [42, 82, 83] that the size of wounds and the infliction of a second wound significantly affects the rate of healing of the first wound, pains were taken to ensure that all biopsies were consistently as close as possible to ½-1 inch in length and ¼ inch wide at the greatest width. If 2 biopsies were made on the same side, the first was always taken towards the cranial end of the initial incision and the other, at a later date, towards the caudal end. Biopsies were usually taken from each side alternately.

At the end of the experiment the animal was sacrificed by an intravenous injection of 6 to 10 cc. of 1/6 molar magnesium chloride solution. Biopsies were finally taken in the middle of the original wound, as well as from the wounds of the previous biopsy sites. Thus, if the wound on both sides had originally been made on day 0 and biopsies had been performed on day 4 of the experiment on the left, and on day 10 on the right side, and the animal subsequently sacrificed on day 15, then on day 15 biopsies were obtained from wounds that were now 15, 11 and 5 days old respectively. One animal thus frequently yielded biopsies from incised wounds that were 4, 5, 10, 11 and 15 days old. In the above example the main discrepancies between biopsies performed on the fourth and tenth days and those obtained at the time of death (as biopsies of the biopsy sites) were that the planes of histological section of the latter were at right angles to those of the former.

(*b*) *Rats.* Rats, weighing between 125 and 200 g. were used. Under ether or intraperitoneal nembutal anaesthesia, 2 types of wounds were made on each of the rats. Wound *A* was an *excised* wound about ½ inch in diameter made with sharp, curved scissors in the midline of the carefully shaved abdomen, just below the xiphisternum and including all layers down to deep fascia. Wound *B*, made on the same rat, at the same operation, was an incised wound 1 inch long, immediately to the right of the midline, extending from just below the umbilicus almost to the inguinal ligament. The incision was carried through all layers, including the peritoneum. The peritoneum and muscle sheath were closed, together, by a single continuous 4-0 catgut suture. The skin and subcutaneous tissue were then also closed, together, with another continuous 4-0 catgut *everting* suture. In the earliest experiments, both wounds were covered with sterile *tulle gras* and lint and the dressing was maintained in position by a 1 inch wide plaster bandage about 14-16 inches long.

In subsequent experiments the wounds were left completely undressed. This procedure was adopted because many of the rats chewed off the plaster bandage case—despite the painting of the plaster with picric acid or bitter aloes (*Aloe capensis*); hence different animals retained dressings for different times. We found, in animals which had chewed off their plaster and dressing within the first 24 hours after operation, that the wounds did not become septic and that they seemed to heal more rapidly than did the dressed wounds. Moreover, in accord with general experience in laboratories, and particularly that recorded by Localio *et al.* (1943a), we found the rat remarkably resistant to sepsis. Furthermore, the dressing frequently adhered, particularly to the excised wound, and no matter how much care was taken in removing the *tulle gras* and lint, portions of the clot and/or the granulation tissue and new epithelium were often removed with the dressing. As a result, histological comparisons of wounds in different animals, or in animals treated in different ways, became difficult and unsatisfactory. All dressings were therefore omitted from rats and the findings under these conditions are reported below. At the end of the experimental period rats were sacrificed in an ether bottle and the entire wound was gently excised, together with a liberal margin of surrounding tissue.

In another experiment abdominal incisions 2 inches long were made in a further series of 35 rats. The animals were sacrificed by an overdose of ether, and the entire wound plus the surrounding tissue was removed *en bloc*.

The sutures were not removed at any stage of the animal experiments. All biopsies on animals, as well as specimens taken *post mortem*, were performed by one of us. The entire biopsy specimen was gently spread on to

a piece of cardboard, to keep it in position during fixation and subsequent processing. Only after a minimum of 2-3 days' fixation was a suitable portion of the specimen trimmed with a sharp knife, dehydrated and embedded in wax. The remainder of the tissue was kept in formalin for section with a freezing microtome.

All tissues were fixed in 20% formalin. Frozen sections stained with Sudan IV and haematoxylin were made of many of the specimens from rats. All the remaining specimens were dehydrated through benzol and embedded in wax. A long ribbon of approximately 100 *serial sections* was cut from all embedded material. Between 3 and 10 serial sections (depending on the size of the specimen) were mounted on a 3 × 1 inch slide. In all, between 10 and 15 slides were prepared of each specimen, all the slides being numbered serially. In effect, therefore, all specimens examined were serially sectioned, albeit the staining for each slide of the series was not the same.

The following stains were used: Mallory's triple, Weigert's and/or Verhoeff's elastic tissue stains, and haematoxylin and eosin as nuclear or cytoplasmic counterstains; Heidenhain's iron haematoxylin; Wilder's reticulum stain with safranin as a counterstain; Mallory's phosphotungstic acid haematoxylin; Ponceau-2R light green method;[37] Periodic acid—Schiff for mucopolysaccharides and other carbohydrates;[50] Thionin and toluidine blue for metachromatic staining; Unna's safranin and wasserblau and Unna's polychrome methylene blue—the 2 latter as detailed by Hall and Herscheimer.[37]

The first 4 stains were applied routinely to all slides, each slide in the series being stained by a different method. At least 8 slides were prepared of each specimen in this way and frequently 15 or 20 slides were made from a single block. As all material was serially sectioned, it was difficult to maintain the precision of mounting possible with individual sections. Furthermore, the presence in the blocks of the hard silk or catgut sutures frequently resulted in some scoring, tearing or folding of the sections, thus making unavoidable photographs which were not of the highest standard and which had one or other defect, as portrayed in Figs. 5, 8, 18 and 35.

All observations here recorded were gleaned from a study of at least 30, and frequently from 100 or more, *serial* sections. The study of serial sections in this type of investigation is virtually indispensable, for reasons outlined in a previous publication,[27] as well as for reasons related specifically to the study of the dynamic three-dimensional processes which supervene in the healing of incised wounds.*

OBSERVATIONS

Since many aspects of wound healing have been documented fully in the literature, our descriptions will be confined to original observations, or ones which differ from the conventional views on the healing of uncomplicated incised and sutured wounds, such as those made in all standard operative procedures.

To our knowledge there is no account, or even reference, in the literature available to us about the reactions of the *epidermis* to sutures. Our observations will therefore be presented under 2 main headings:

1. The healing of the incised wound.
2. Reactions to sutures and to suture needle puncture wounds in the epidermis.

1. HEALING OF INCISED WOUNDS

As indicated above and in Table I, one healthy human female volunteer provided biopsy specimens of an experimentally induced incised wound made on 2 separate occasions. The first biopsy specimen was taken 24 hours after the original operation. Despite the great care taken to ensure precise apposition of the cut edges of the wounds, nevertheless histological examination of this and the other 24-hour biopsy specimens revealed that the 2 epithelial edges had folded inwards. This was a constant finding in all the biopsy specimens taken at all periods during this study, both in animals and in Man. So constant was this finding and so intimately did it appear to be bound up with the subsequent course of the healing process, both in the epidermis and in the dermis, that it would seem to be a normal phenomenon during the course of the healing of an incised wound and quite unrelated to the precision of the operator's apposition of the cut edges. In fact, this infolding of the epidermis was regularly seen even when *everting* sutures had been used to close the wound, as was the case in rats.

At *24 hours* after the operation the site of incision in the epidermis was filled with a small blood clot, together with some necrotic dermal and epithelial material. The line of incision in the dermis itself was distinguishable only as a narrow pink, purple or orange-stained line extending to subcutaneous fat. Blood or clot were not detectable in the dermal portion of the wound. Even 24 hours after the wound had been made there was clear-cut evidence of *epithelial* response, shown not only by the inversion of the 2 cut edges of the epidermis, but also by the following:

(a) Hyperplasia of the neighbouring epithelium for a distance of approximately 1.0 mm. on either side of the incision. The epidermis over this region increased its thickness from some 6-8 layers in the

* The authors wish to thank Mr. R. A. Mansfield for developing and printing all the photomicrographs and Dr. E. E. Rosenberg for surgical assistance in the experiments on rats.

normal skin of the area studied, to 10-12 layers in the zone of reaction.

(b) A change in the arrangement of the epidermal cells, with the appearance, near the site of injury, of tall columnar epithelial cells occupying at least the bottom 2 layers of the epidermis.

(c) Mitotic figures at the wound edge.

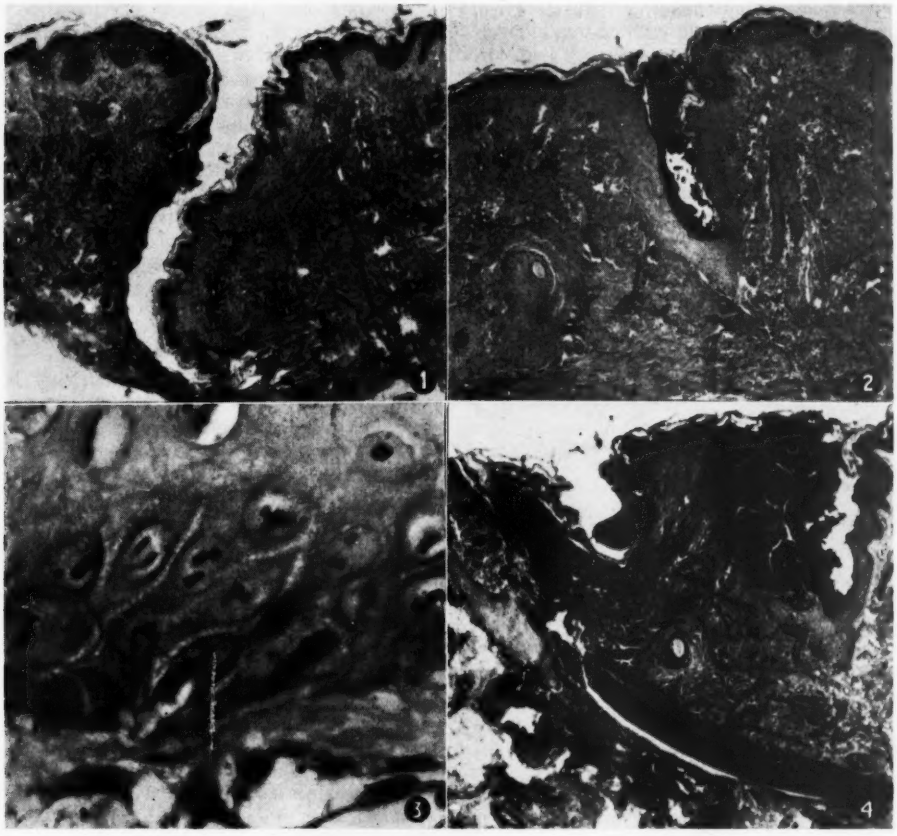
The changes in the associated *dermis* were:

i. The accumulation, in the stratum papillaris of the dermis, of lymphocytes and neutrophils for a distance of approximately 0.25-0.5 mm. on either side of the incision.

ii. The regular occurrence of alteration in the morphology and staining reactions of the collagen fibres, as described in detail below.

iii. Round cell infiltration in the transected subcutaneous fat.

At *48 hours* after operation (Fig. 1) the thickening of the epithelium in the zone surrounding the wound is clearly apparent, as is also the broadening of the rete pegs. Within the incision itself the epithelium is distinctly hyperplastic, with the broadening rete pegs



Note: Magnifications are those of the photomicrographs before their reduction, in printing, to about $\frac{7}{8}$ of the original size.

Fig. 1. Human healing incision, second post-operative day, showing inversion of epithelial edges, hyperplasia of neighbouring epithelium (× 43).

Fig. 2. Human healing incision, fourth day, with union across the incision, of hyperplastic epithelium growing from both cut edges. Note absence of fibrosis in dermal part of the incision (× 43).

Fig. 3. Human: fourth day. Actively mitosing epithelial cells from base of inverted wound edges seen in Fig. 2. Prickle cells extend to dermal fibres without any intervening clot or cellular tissue debris (× 1000).

Fig. 4. Human: fourth day. Inverted epithelium in incision, at right; spur of epithelium growing along suture at left. Two apparent epithelial pearls representing transected hyperplastic hair follicle. 'Elastotic change' of collagen in traumatized area well marked. No fibrosis around suture (× 43). (For details of reaction to suture, see also Fig. 23). Weigert's elastic stain.

straightening the epidermal-dermal junction. In Fig. 1 the cut edge of the epidermis on the left side shows marked hyperplasia of the epithelium which is spreading deep to both the clot and the damaged connective tissue fibres. This invasive growth of the epithelium is extremely active during the first 4-5 post-operative days. The regenerating epithelium constantly develops deep to the blood clot (when this is present in the incision) to *establish direct contact with the undamaged dermal fibres*. There is, at this stage, no evidence of fibroplastic proliferation along the edges of the incision in the dermal portion of the wound. We have found in all material studied that the stratum reticularis, in particular, remains remarkably inactive for 3-4 days. Fibrosis is, however, already actively in progress in the incised subcutaneous muscle in the rabbit and in the subcutaneous fat, which is infiltrated with round cells apparently derived from the blood (Figs. 37A, B).

By the *4th day* (Figs. 2 to 7) the gap in the epithelium, caused by the incision, seems in some sections to have been completely bridged by the regenerated epithelium (Fig. 2). In areas such as that depicted in Fig. 2, it can be seen that not only is the epidermal gap bridged, but the regenerating epithelium has also undergone marked thickening within the V-shaped depression caused by the epidermal infolding. While the rete pegs are still visible within the zone of repair in Fig. 1, they have disappeared in the specimens portrayed in Figs. 2, 4 and 5. Once more the observation is made that the regenerated epithelium lies *directly* on the cut surface of the intact and seemingly inert underlying dermis, within the line of incision (Figs. 2-7, 37).

Examination of the characteristics of the regenerated epithelium, particularly at the base of the 'V', reveals numerous mitotic figures at the 4th day (Fig. 3), and the presence of

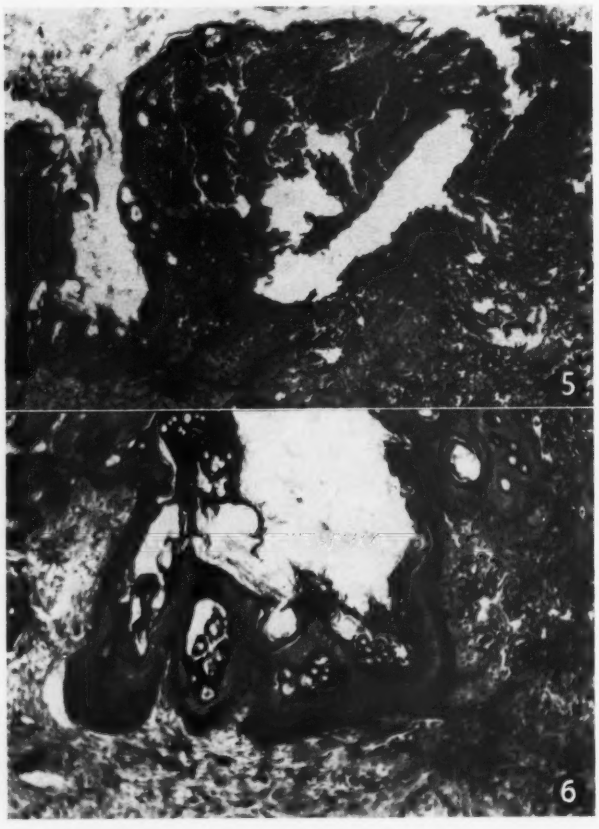


Fig. 5. Rabbit: fourth day. Hyperplastic irregular epithelium within inverted incision, at left; at right, commencing epithelial invasion along line of suture which has torn out during sectioning (× 43).

Fig. 6. Rabbit: fourth day. High power of hyperplastic irregular epithelium within inverted incision, shown at left of Fig. 5. This is the point of junction of the regenerating epithelium bridging the incision. Note also some early fibrotic reaction immediately below epithelium (× 98).

prickle cells extending right down to the dermal fibres. This finding of mitotic figures in the epithelium in the zone of repair is contrary to that generally recorded in the literature.[2, 40, 41, 52, 53] We have, of course, encountered numerous mitotic figures in the epidermis adjoining the zone of repair, in the manner described by other authors and we have even accumulated unusual evidence, from the study of healing wounds in pigmented Negro skins, that active epithelial migration can and does occur, especially during the repair of excised wounds. We feel it desirable, however, to stress here our finding of numerous mitotic figures *in the epidermis within the zone of repair* of an *incised* wound.

By the 4th day the clot and tissue debris (seen at the site of injury, 24 hours post-operatively) lie superficial to and in the gap between the epithelialized walls of the wound, and clearly separated from the dermis by the now almost intact line of stratified epithelium. This clot may, therefore, even at the 4th day, be regarded as having been shed, especially since it is very loosely adherent to the keratinizing surface of the new epithelium. Keratinization of the regenerated epithelium, within the healing wound, is proceeding actively by the 4th day, but without the associated presence of a stratum granulosum. In dermatological parlance, this may be regarded as a form of parakeratosis. This reaction was observed in both the human and the animal material.

The mode of union of the epidermis, regenerated from the 2 sides of the incision, has received scant attention in the literature. This is probably because serial sections of wounds have very rarely, if ever, been reported on. It is only by the use of this procedure that it is possible to establish, with any degree of precision, the location of the original break in the surface epithelium resulting from an incision. This line of union is clearly portrayed in Figs. 5-7, where, in the depths of the V-shaped dip which characteristically supervenes in incised wounds, the great irregularity in the organization of the regenerating epithelium is well shown. Fig. 7 represents a serial section of the same specimen as that shown in Figs. 5 and 6. The relations between the incision, the site of suture entry and the disorganization in the regenerated epidermis, are shown in Fig. 5, whereas in Figs. 6 and 7 details are shown of the unusual arrangement of the epidermis at the site of union between the epithelium regenerating from both sides of the incision. In Fig. 6 where the 2 regenerating epidermal edges meet there seems to be a 'head-on collision' with a consequent heaping up of new epidermis, both into the V-shaped gap on the wound surface, as well as downwards into the underlying dermis. The excessive epithelium undergoes an unusual type of internal keratinization (Fig. 6) and also sends characteristic spurs of cells into the immediately underlying dermis (Fig. 7). This process of internal keratinization results in a remoulding of the epidermis and the elimination of excessive epithelial cells. The intensity of epidermal activity, even as early as the 4th day of the healing process, as opposed to the dormancy of the underlying dermis, is clearly shown in Figs. 6 and 7, where the accumulation of fibrin and of small pockets of apparently freshly shed blood are constantly encountered within the line of incision; fibroblasts are still extremely rare except in the subcutaneous fat and in the severed muscle. If the epithelial invasions of the dermis have already become well marked, as may occur in the rabbit by the fourth day, fibrosis in the immediately sub-epithelial layer of the dermis may also be well advanced. We have confirmed Hartwell's observations [38-41] that the most actively fibrosing tissue, during healing, is the traumatized fat, and that the transacted collagen remains inert.

The epithelium adjoining the zone of repair is distinctly thickened by the fourth day and shows a change in the organization and width of the rete pegs (Fig. 8). Mitotic figures are numerous, tall columnar epithelial prickle cells, as described in the 24-hour specimen, are encountered throughout this portion of the epidermis, and the rete pegs seem to be invading the dermis which has now become quite cellular, due to the infiltration of round cells. These reactions in the epidermis at the site of injury are complicated by the striking changes which supervene, both in the dermis and in the epidermis around the sites of entry and exit of the suture needle, as well as around the suture itself (Figs. 4, 5, 8, 21-25, 37). For details, see below).

While there is usually little evidence of regeneration of connective tissue in the dermis at this time (4th day), there are nevertheless some changes in the dermis which merit description. In particular, as shown in Figs. 4 and 8, the collagen fibres have undergone a change both in their morphology and staining reaction. The most striking changes in morphology are the thickening of these collagen fibres, which also become homogeneous

(i.e. lose their longitudinal wavy striations). These altered fibres acquire a particular affinity for certain basic dyes, such as safranin, as well as for the majority of stains which are generally considered to be specific for *elastic* fibres. These alterations in the collagen fibres are extremely constant in the zone of trauma. They are probably equivalent to the changes in collagen described by other authors as 'fibrinoid degeneration'. These alterations in the collagen fibres, as well as the increased cellularity of the stratum papillaris of the dermis, both within the line of injury and in the immediately abutting areas, seem to play an important rôle in promoting the alterations in the metabolism of the epidermis accompanying epithelial regeneration.

Very striking changes in the epidermis are seen in specimens removed on the *6th and 7th post-operative days.* Once again, the inversion of the epithelium into the incision was seen in all specimens studied. The epithelium was very markedly thickened over the entire area under study, i.e. the wound itself, the sites of suture entry and exit, as well as in the neighbouring epidermis for a distance of 2-3 mm. around the injury. There were numerous mitotic figures in sweat gland ducts, in hair follicles and at the necks of sebaceous glands located in the skin adjoining the wound. Within the line of the incision itself the epidermis was mitotically extremely active and numerous secondary epithelial spurs were found invading the underlying dermis (Fig. 37). Serial sections reveal that these secondary epithelial spurs are developing radially around the entire circumference of the epithelium lining the V-shaped depression now constituting the line of incision. This view is supported by an examination of Figs. 9 and

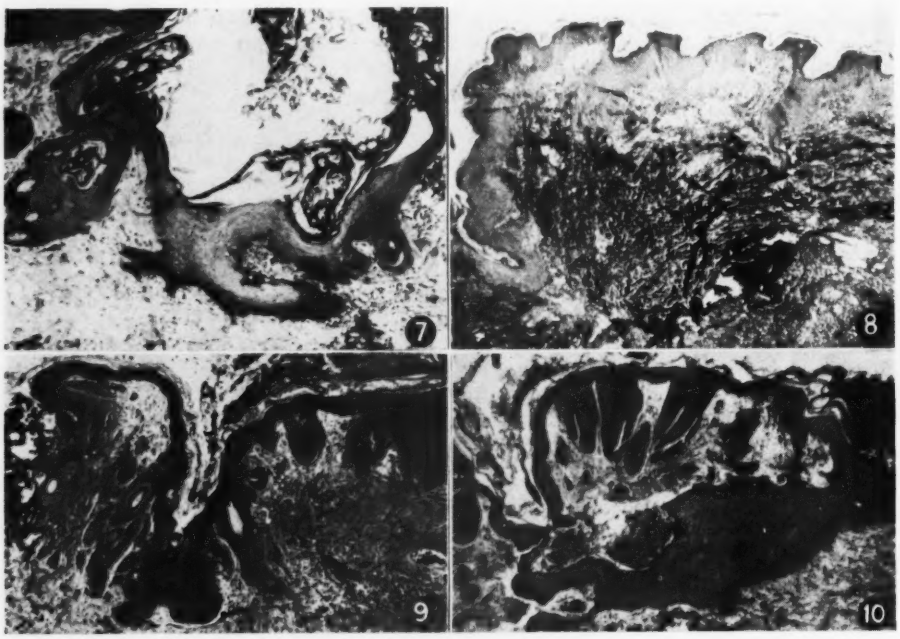


Fig. 7. Rabbit: fourth day. Serial section to Fig. 6 showing epithelial spurs invading underlying dermis, with early internal keratinization and pearl formation within incision (× 98).
Fig. 8. Human: fourth day. Hyperplastic epithelium neighbouring incision (shown at left) with elastotic change in collagen due to trauma between suture (lower, centre, black oval) and incision. Weigert's elastic stain (× 43).
Fig. 9. Rabbit: sixth day. Incision with numerous secondary spurs undergoing internal keratinization (centre) invading dermis. At right, entry point of suture with related collagen and epithelial changes; at left hyperplastic epithelium with secondary spurs related to suture exit. Note fibrosis related to latter (× 43).
Fig. 10. Rabbit: sixth day. Serial section to Fig. 9 showing junction between epithelial reactions within inverted wound and related to suture (at left, Fig. 9). Hyperplastic mass of epithelial bar so formed is undergoing internal keratinization (black oval in centre of epithelial bar) (× 43).

10, which are quite widely separated serial sections of the same specimen. These epithelial spurs thicken, being constituted of up to 10 or 12 layers of cells, with prickle cells predominating, as already shown in Fig. 3. These fairly thick spurs soon undergo internal keratinization, forming epithelial pearl-like structures, as described above. (See also Fig. 37D.) However, these pearls differ from those encountered in malignant epidermal lesions in that, for some time, the interior of most of these spurs communicates with the surface through duct-like structures which now resemble hair follicles. (See both sides of V-shaped depression in Fig. 9.) The internally keratinizing spurs may rapidly undergo differentiation into abnormally large new hair follicles, particularly in animals. This new development of hairs is regularly associated with the differentiation, in the walls of the follicle, of clear lipoid-containing cells, indistinguishable from sebaceous glands and particularly well shown in Fig. 11.

One cannot state dogmatically that there has been neogenesis of both hair and sebaceous glands in hairy animals like rabbits and rats. However, there seems to be very little doubt of the neogenesis of these structures in human material, especially as the normal skin of the area studied is sparsely hirsute. Furthermore, there appears to be a distinct concentration of hairs and sebaceous glands in the immediate

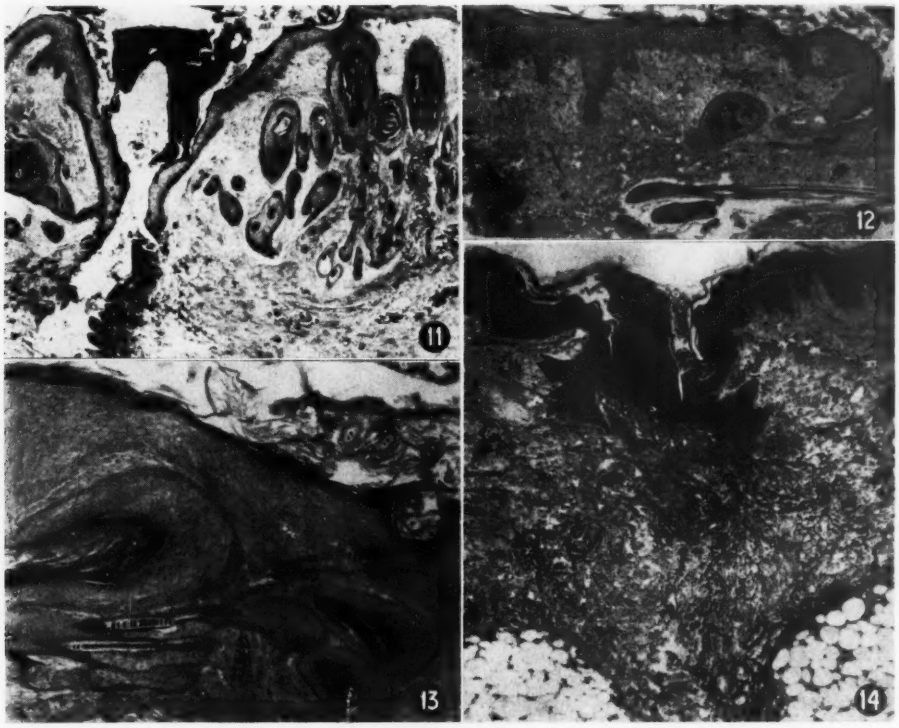


Fig. 11. Rabbit: seventh day. (Treated with British anti-lewisite) showing hyperplastic epithelium invading along suture with massive epithelial pearl formation from secondary spurs invading surrounding dermis from cuff of epithelium about suture and wound (changes just visible at right of photographic field) (× 43).
Fig. 12. Rabbit: eighth day. Repairing wound at right with formation of internally keratinized epithelial pearls and abnormal giant hair follicles. Hyperplastic epithelium at left is related to suture (× 43).
Fig. 13. Rabbit: seventh day. Massive epithelial hyperplasia with hyperkeratosis of surface, internal keratinization of secondary spurs and formation of giant abnormal hair follicles at side of incision. Note fibrosis in dermis related to epithelial pearls (× 98).
Fig. 14. Human: eighth day. Marked epithelial hyperplasia in incision as well as secondary invasion of dermis. Dark area in line of incision below epithelium extending to fat is shown in Fig. 15 (× 43).

vicinity of the incision and the suture puncture wounds. Even in rabbits and rats the hair follicles and their contained hairs seem unduly large, compared with similar structures usually seen in the normal skin. Moreover, these structures are very irregularly arranged and are peculiarly related to the site of incision (Figs. 12 and 13). The disposition and the morphology of the sebaceous-like cells in these apparently new follicles seem indistinguishable from that recorded in the developing sebaceous glands during the early post-natal period of the rat (Parnell, 1949). Under certain experimental conditions (e.g. when British anti-lewisite is administered during the period of healing) the formation of epithelial pearls and new sebaceous glands, within invading epithelial spurs related to the incision, may become so marked that, on occasion, the reaction may simulate early, benign intra-epidermal neoplasia (Fig. 11). It is our finding that this neogenesis, at least of the hair follicles and sebaceous glands, is a constant reaction during healing of epidermal damage in incised wounds. This reaction has been recorded in photomicrographs by many other authors who have usually regarded it as an artifact (see figures and legends in article by Baxter *et al.* [6]) While this paper was in the press a paper by Breedis (Cancer Research, *14*, 1954, 575) established the neogenesis of hair fibrils and sebaceous glands as a constant occurrence in scar epithelium in the rabbit.

Apart from the changes in the epidermis within the line of repair, the subsequent behaviour of the epidermis during the healing of a sutured incised cutaneous wound is complicated by the epidermal reactions at the sites of suture needle punctures and in relation to the suture itself. The epidermis actively invades the dermis along the line of suture from the points of entry and exit of the needle (Figs. 11, 21-24, 37B-37E). These invading epidermal bars around the suture may and frequently do unite with the epidermal invasions in the line of the incision (Figs. 10, 16-19, 37C). The subsequent reactions of the epidermis as well as the final line of epithelium covering the site of injury is profoundly affected by this union of invading epithelial bars originating at different sites in and around the wound. This point is stressed because, from an examination of all the figures here presented, it will be apparent that *the greatest difficulty may be experienced in distinguishing the reactions encountered along the suture line from those within the incision itself*. This is particularly the case, of course, if a study is being conducted on *single* sections of sutured wounds. In most instances we found it possible to distinguish between the reactions to the suture needle punctures and those within the incision only after careful study of serial sections, particularly in specimens removed after the 6th post-operative day.

On the *6th and 7th post-operative days* there are still only moderate reactions along the incision in the dermis. A distinct fibrinous (*not* fibrous) line, clearly showing the direction of the incision, is usually visible in the dermis. Very little cellular exudation is detectable in the dermis about the incision itself, although there is already well-advanced fibroblastic regeneration and fibrillogenesis in the more deeply located transected fat and muscle. The degree of connective tissue reaction at this stage is clearly much more vigorous and further advanced in the damaged muscle and fat than in the dermis. The tensile strength of the incised wound at this stage is probably determined in large measure by the repaired hyperplastic epidermis, the invading anchor-like spurs of epithelium and immediately subepithelial fibrosis, together with the well-advanced fibrosis within the adipose and muscle layers and around the suture.

We have completely confirmed Hartwell's views that fibrosis during cutaneous wound repair proceeds most actively in the subdermal fat. We also agree with him that the fibroblasts are derived primarily from the haematogenous round cells which infiltrate the traumatized fat shortly after the infliction of injury, more especially as the cut ends of the dense dermal collagen remain remarkably inert. Fibroblasts seem to develop from the round cells which infiltrate fat and which encircle invading epithelial spurs. The fibroblasts from these 2 loci appear to invade upwards and downwards into the portion of the incision which traverses the stratum reticularis of the dermis.

These views are further substantiated by our findings, both in human and in animal material, at the *8th post-operative day*. At this time the hyperplasia of the epidermis in and around the line of incision is once more apparent, as are the spurs of epithelium invading the dermis (Fig. 14). In this latter picture there would appear to be an intense fibroblastic response in the subdermal fat and also just below the hyperplastic epithelium. Closer examination soon reveals that, at this time, there is only a well developed *fibrinous* exudate in the dermal part of the incision (Fig. 15). The most marked fibroblastic activity at this time is

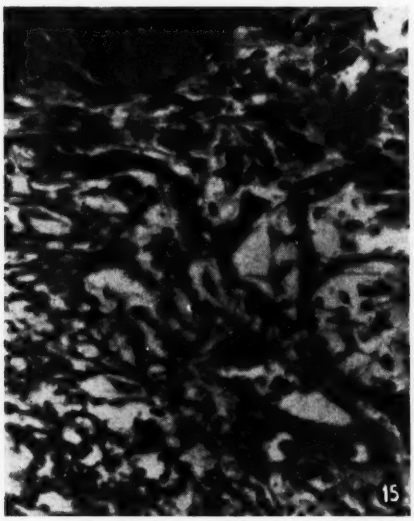


Fig. 15. Human: eighth day. High power of epithelial-dermal junction in line of incision. Epithelium just visible above, with early sub-epithelial fibrosis, below which lies fibrinous exudate with red blood cells and very few fibroblasts (× 440).

seen at the sites of 'invasion' of the hyperplastic epidermis into the dermis (Fig. 15) and in the fat. In both these locations the fibroblasts seem to be derived from round cells. Particularly in animal material, the association between invading epithelial spurs and the onset of fibrosis in the subepithelial portion of the dermis is always impressively consistent. Indeed, it seems possible that intra-dermal fibrosis (*not* fibrosis in fat or muscle) may be initiated by virtue of the round cell infiltration evoked by the invading epidermis. This possibility is supported by the fact that fibrosis does not easily supervene in granulating excised wounds until epithelium covers the surface of these granulations.[27] Fibroblasts are extremely rare in the centre of the cone-shaped fibrinous exudate mentioned above (Fig. 15); along the *edge*, especially where this abuts on the intact dermis, there is an active invasion of the exudate by vascular buds, by round cells and by monocytes which soon differentiate into fibroblasts. Most of the round cell-like structures seen in Fig. 15 are fresh red blood cells which have entered the exudate, either by recent diapedesis or, what is more likely, from trauma to the thin-walled new blood vessels surrounding and invading the fibrin, at the

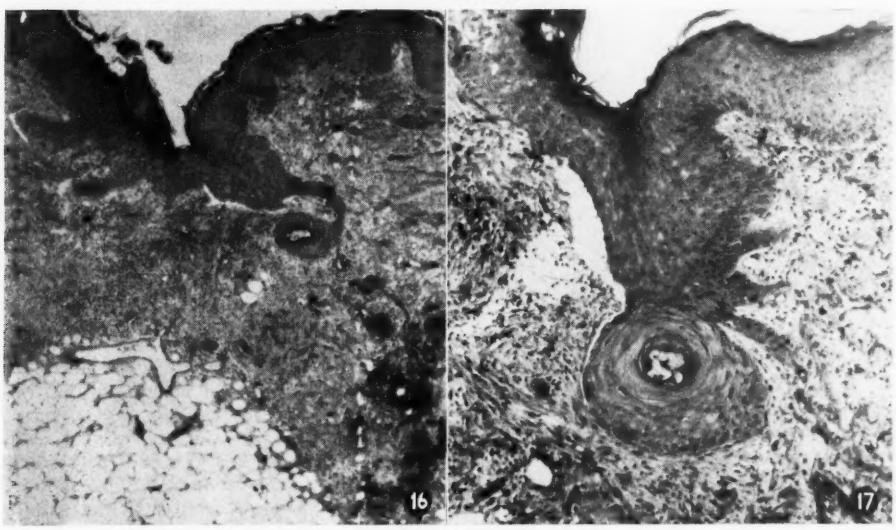


Fig. 16. Human: tenth day. Epithelial hyperplasia within inverted incision line with secondary spurs and junction of epithelial reaction at incision with peri-sutural epithelial cuff (× 43). (See also Figs. 17, 30 and 31 for details of this specimen).

Fig. 17. Human: tenth day. Serial section to Fig. 16. Hyperplastic epithelium with secondary spurs, in incision, uniting with peri-sutural epithelial cuff containing suture in transverse section. Note fibrosis around latter extending also to left (× 98). (Compare with Fig. 30).

time of biopsy. The generally propounded view that fibroblasts originate from activated fibrocytes in the stratum reticularis of the dermis could not be substantiated. This portion of the dermis remains remarkably inert and is also the last to heal. Even when this region does fibrose, it does so by invasion of cells and capillaries from the adipose tissue below and from the sub-epidermal new fibrous tissue above.

Fibroblast population and fibre formation increase rapidly between the 8th and 10th post-operative days (Figs. 16-18). It is our impression that this sudden and rapid invasion of the site of incision by new connective tissue elements may be related immediately to the heavy round cell infiltration about the epithelial wedges growing into the dermis, both at *within* the healing incision, at this time, they are rare among the dense fibre bundles of the dermis on either side of the incision. These mitosing fibroblasts, migrating along the protein threads in the *new* fibrinous exudate within the incision, seem to be derived from haematogenous round cells.

Between the *8th and 15th post-operative days* the changes, both in the epidermis and in the dermis, are once more distinctly affected by the reactions to the suture and to the suture needle. This is apparent in Figs. 16-20 and in Figs. 34-36. Figs. 16 and 17 are serial sections of the same wound, showing the union between the epidermal invasions at the incision and the thick epidermal cuff which is developing along the suture (Fig. 37C). These spurs of invading epidermis are distinctly shown as

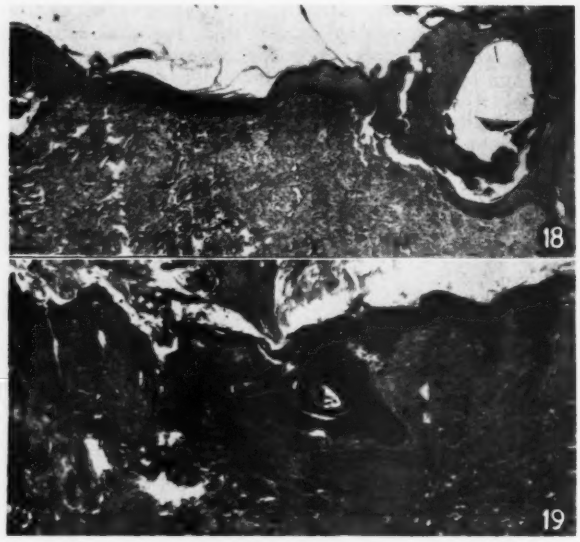


Fig. 18. Rabbit: eighth day. New line of epithelium formed from *lower* portion of epithelial bar joining epidermal reactions at incision and about suture. The tissue *above* the line of internal keratinization within this bar (see Fig. 10) together with the suture is being sloughed, with the resulting remoulding of the scar (× 43).

Fig. 19. Rabbit: twelfth day, showing remoulded scar resulting from junction between epithelial reactions at wound (near right edge) and about suture (at centre), with subsequent reactions as indicated in Fig. 18 (× 43).

the site of the original incision and along the suture (Figs. 37B-37E). This possibility receives some support from the following evidence:

(a) Apart from the fat and muscle, fibroblasts are most numerous and, by this time, are regularly related to the round cells around both the suture and the invading epithelial spurs. In serial sections the suture seems to be encased within a cellular fibrous tissue sheath (Figs. 24, 25, 31, 32, 36, 37B, 37C);

(b) Formation of mature fibroblasts and the deposition of fibres in granulation tissue within an *excised* wound seem to be directly related to the growth of epithelium across the granulations, either from a graft or from the edges of such wounds.[27]

While mitosing fibroblasts are common is also the fibroblastic reaction, particularly around the suture and its associated epithelium. In Fig. 18 the epithelial invasion within the incision is seen at the left of centre, and that related to the suture entry, towards the right. It can be seen that the bars of invading epidermis, commencing at these 2 separate sites, have united by growing through the dermis, and that the intervening tissue is in the process of being shed, consequent on the internal keratinization of the united epidermal bars. This results in considerable distortion and remoulding of the surface of the wound (Figs. 18, 19).

Associated with these changes is a further secondary epithelial invasion of the residual underlying dermis (from the new line of surface epithelium), the formation of numerous internally keratinized cysts, hair follicles, etc. (Figs. 13, 15, 16), as well as a marked fibrosis of the dermis proceding from above downwards and from the fat, below, upwards. The extent of these latter reactions, as well as the depth to which the epidermal bars may invade the dermis along the suture, is shown in Fig. 20, where an epithelial lining of the lower

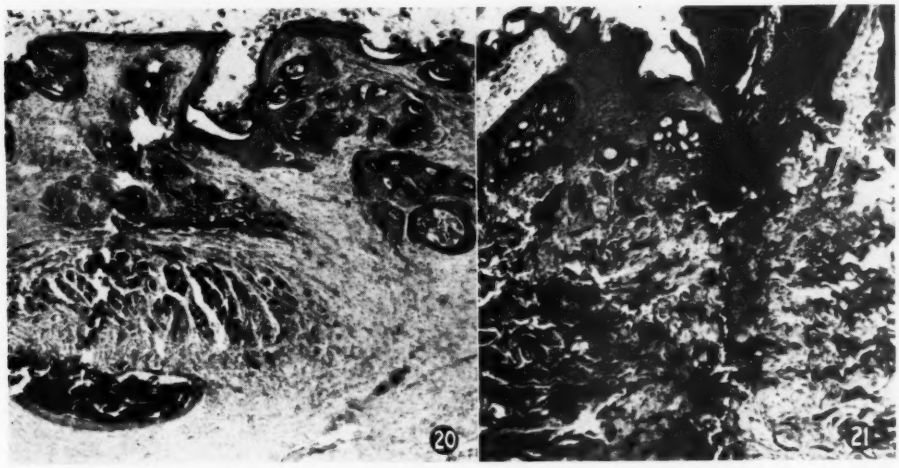


Fig. 20. Rabbit: fifteenth day. Epithelial reaction within inverted incision (top centre) and secondary pearl formation about incision. Below this lies an area of panniculus carnosus and below this again a sickle-shaped suture, lined on its *deep* surface by stratified epithelium. Fibrosis along original incision extends towards right (× 43).
Fig. 21. Rabbit suture reactions: fourth day. Section passes centrally through suture-entry crater; marked epithelial thickening and early invasion along suture on both sides. Alteration in staining of surrounding collagen, blood along suture track without associated fibrosis (× 98). Weigert's elastic stain.

border of the suture can be seen lying deep to the panniculus carnosus (Figs. 37C, 37D).

Hartwell[38-41] has repeatedly drawn attention to the following phenomena:

(a) The regenerating epidermis regularly grows in contact with a solid base.
(b) The cut edges of the dermal collagen remain inert throughout the healing process.
(c) Fibrosis is closely connected with adipose tissue and the 'macrophages', derived from blood lymphocytes, which infiltrate the fat.

We can confirm all these meticulous *observations* by Hartwell, although we are not in complete accord with him in his *views* about the nutrient functions of the fat or the purely 'mechanical' functions of dermal collagen. We have been impressed repeatedly by the absence of mitotic figures in the fibrocytes lying at or near the edges of the transected dermal collagen. We agree with Hartwell and with Moschcowitz[66] that the blood lymphocytes play a major rôle in the formation of the first fibroblasts which appear during healing. Whereas mitotic figures were not encountered in the pre-existing dermal fibrocytes, considerable evidence of multiplication was seen among fibroblasts, apparently derived from blood cells and perithelial tissue. These mitotic figures in fibroblasts were usually found *deep* within the areas of fibrosis and *not* at the edges of the transected dermal fibres.

We are also in accord with Hartwell in his assertion that adipose tissue is rapidly activated to form fibrous tissue after trauma, although, once again, we are not in a position to accept his theoretical explanation to account for this reactivity of fat. However, we have ample evidence from previous studies on tumour formation in connective tissues and lymph nodes, following trypan blue injections[23, 25, 26] and from the study of the pathogenesis of certain types of hepatic fibrosis, that fat may rapidly become highly cellular. The fate of such highly cellular fat is entirely dependent upon the prevailing local and systemic physiological states. It is generally recognized that such cellular fat may, and does, rapidly change into erythro- or granulo-cytopoietic tissue (*vide* the rapid transition, in appropriate circumstances,

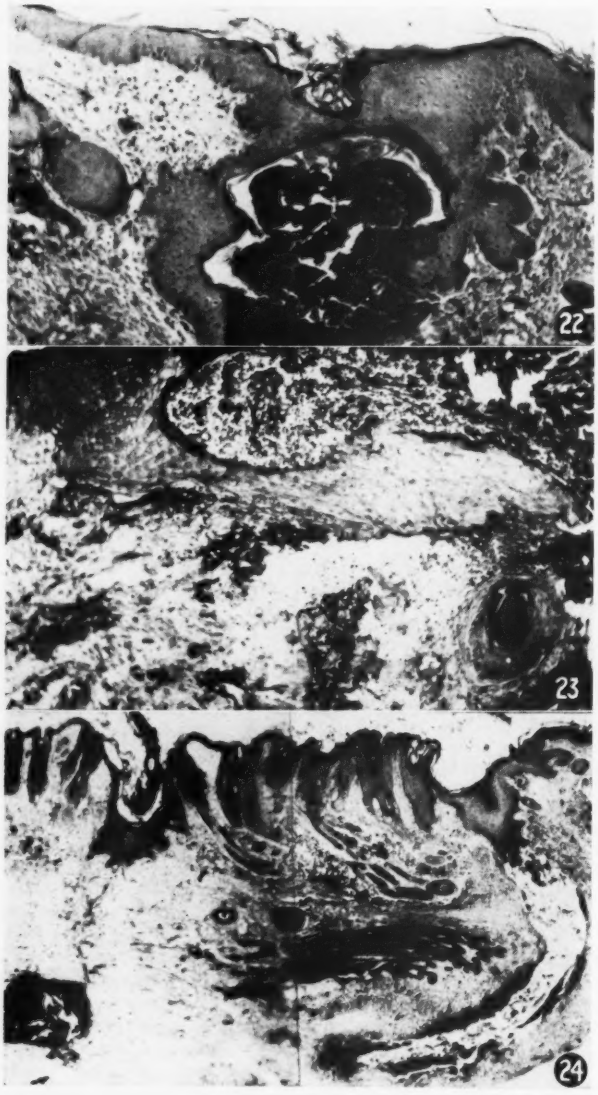


Fig. 22. Rabbit reaction to suture: fourth day. Serial section to Fig. 21 through side of suture puncture wound showing marked epithelial thickening, invasion of epidermis along suture and formation of secondary internally keratinizing spurs and massive keratin formation within suture channel (× 98).

Fig. 23. Human: fourth day suture reactions, with marked epithelial hyperplasia, lateral to suture invasion of epidermis along lower border of suture, joining with hyperplastic transected hair follicle at right. This is a high power of the left edge of Fig. 4 (× 98).

Fig. 24. Rabbit: fourth day wound and suture reactions. Montage of 2 neighbouring fields taken from same section. At right—epithelial reaction to suture with secondary spurs; suture, having torn out during sectioning, leaves channel surrounded by altered collagen and passes through the panniculus carnosus. At left—depression with associated hyperplasia in epithelium, represents incision, below which the very mild fibrosis in the dermis can be seen. Note marked similarities between reactions to suture and to wound (× 25).

of 'yellow' or fatty marrow into 'red' or haemopoietic marrow in long bones) or into lymphoid tissue, into fibrous tissue or even into connective tissue tumours of different types. The profound and rapid changes in fat surrounding lymph node capsules has also received close attention by us in previous studies, where it was shown that such pericapsular fat plays an important rôle in lymph node enlargement.[23, 25, 26] The apparent relationship between fatty change and the onset of fibrosis in the liver should also be borne in mind in a consideration of this problem. We are not unduly surprised, therefore, to find that the subcutaneous adipose tissue plays a major rôle during wound healing.

We would like to add, further, that the *last* zone to become fibrosed is that portion of the incision traversing the stratum reticularis of the dermis. First the fat and transected muscle, then the subepithelial stratum papillaris and finally the stratum reticularis of the

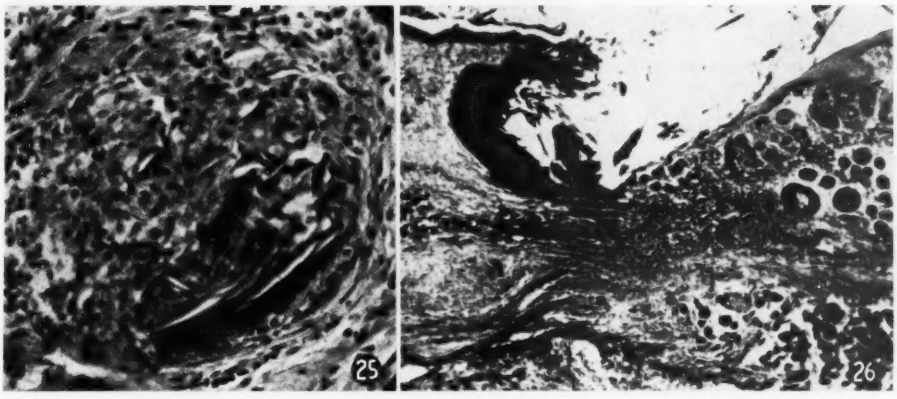


Fig. 25. Rabbit: fourth day reaction to suture deep in dermis, showing peri-sutural leucocytic infiltration and some early fibrosis about this infiltrate (× 220).
Fig. 26. Rabbit: sixth day, showing marked epithelial thickening and alteration in collagen related to suture. Compare with Fig. 8 and note difficulties in distinguishing on a single field, between reaction to suture and reaction to incision (× 43). Weigert's elastic stain.

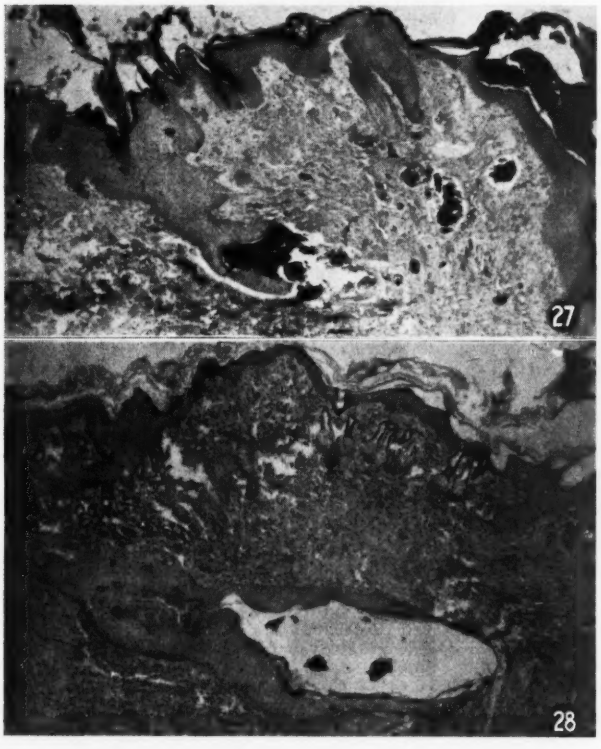


Fig. 27. Rabbit: seventh day. Massive epithelial hyperplasia and invasion extending from *silk* suture, at left, deep into dermis; note paucity of surrounding fibrosis. Line of incision with keratinization within inverted repair zone (top left—centre), epithelial hyperplasia at right related to suture at exit point (× 43).

Fig. 28. Rat: seventh day. Massive epithelial reaction around *catgut* suture, with massive secondary epithelial spurs showing internal keratinization. Thickening of epithelium with broad pseudopeg, towards right of figure, represents healed incision (× 43).

dermis become invaded by the new fibrous tissue. The direction of alignment of the new cells and vessels in the healing stratum reticularis is *in* the line of the incision, i.e. perpendicular to the surface (*not across* the incision) and these new connective tissue elements seem to spread mainly from the fibrotic process in the adipose tissue. They appear last and persist longest in the stratum reticularis.

It is thus apparent that the final phases in the healing of incised wounds, the remoulding of the healed incision, the ultimate fate of the epithelium penetrating the dermis deeply along the suture, and the origin and development of new fibrous tissue, are only determined after the passage of many weeks. The reaction within a site of injury continues for months, and perhaps even for years, after the time when such clean, uncomplicated, incised wounds are generally regarded as completely healed by primary intention.

2. REACTIONS TO SUTURES AND TO SUTURE NEEDLE PUNCTURE WOUNDS IN THE EPIDERMIS

Our findings concerning the reactions of *dermal connective* tissue to silk and catgut sutures do not differ in any way from those described by other investigators.[52, 53, 59] On the other hand, the *epidermal* reactions to sutures and the resulting alterations in the entire healing process encountered in our material, were most striking.

Some indication has already been provided of the alterations in collagen fibres induced by trauma both in the wound and in relation to the suture track (Figs. 4, 8, 11, 24, 26). These changes in the collagen are most prominent between the 4th and 6th post-operative days and are not easily detectable thereafter. In addition, infiltrations of leucocytes, histiocytes and later of fibroblasts and giant cells, were consistently found around those parts of the sutures lying deep in the dermis and well removed from the epidermis. Such peri-sutural cellular infiltrations were well marked even by the 4th day. At this time (Figs. 25, 37) the cellular infiltrations immediately in contact with silk sutures consisted primarily of leucocytes and especially of eosinophils, eccentrically arranged around the sutures. By the 4th day fibroblasts were beginning to appear, primarily around the circumference of the main body of leucocytic cellular infiltrations and apparently derived from the haematogenous round cells. As will be seen below (Figs. 30, 31) giant cells usually appeared much later during the repair process and, invariably, only after the peri-sutural fibroblastic reaction was already well developed.

The response of the epidermis at the suture needle puncture wounds is as rapid and vigorous as that within the incision itself; often even more so. Thus by the 4th day marked thickening of the epidermis at the site of punctures is consistently detectable and this hyperplastic epithelium has already invaded along the line of the suture for some distance

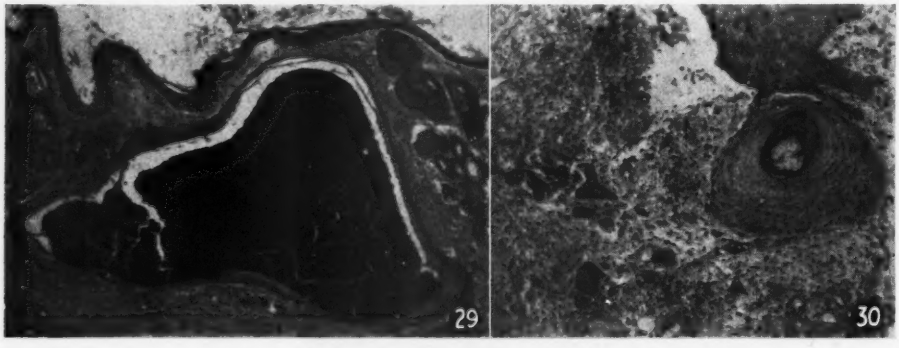
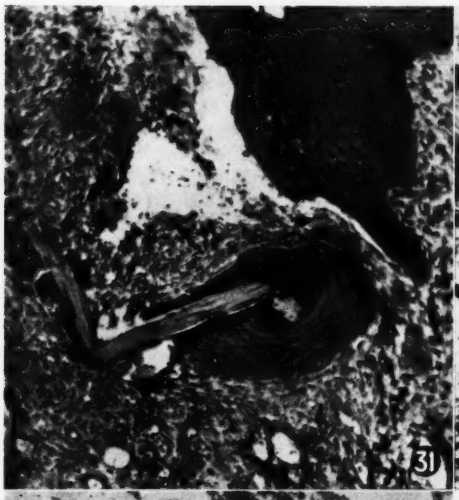
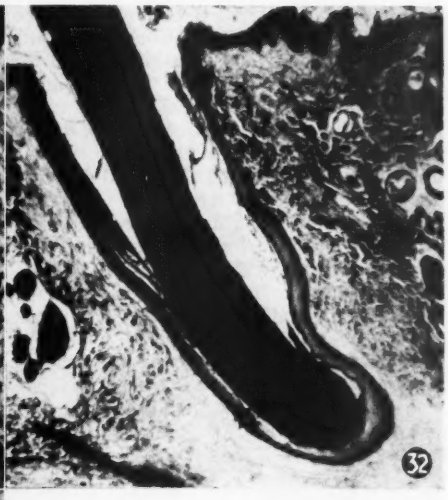


Fig. 29. Rabbit: sixth day. Infection of peri-sutural epithelial reaction with formation of pus pocket lined by stratified epithelium. Note also marked epithelial pearl formation and fibrosis surrounding this infected epidermal-lined pus-containing cyst. Compare with virtual absence of fibrosis about similar non-infected epithelial reaction to suture in Fig. 27 (× 43).

Fig. 30. Human: tenth day. At right, peri-sutural epithelial cuff, uniting with invading hyperplastic epithelium in incision, with surrounding fibrosis. (Compare with Figs. 16 and 17). At left, chain of numerous foreign body giant cells which can be seen in Fig. 31 to be related to the suture and the keratin from the defective peri-sutural epithelial cuff (× 98).





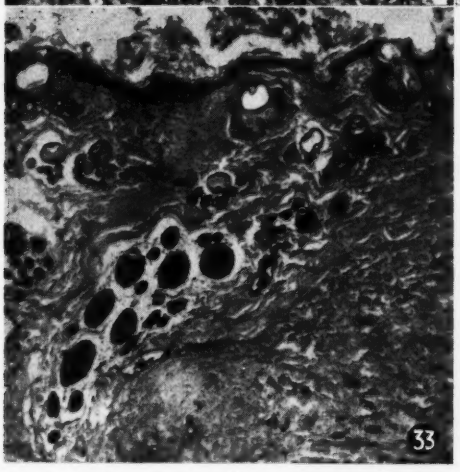


Fig. 31. Serial section to Fig. 30 showing identical peri-sutural epithelial cuff which is defective at one point, resulting in exposure of dermis to both suture and keratin, with consequent marked fibrosis and giant cell formation (× 98). (Compare Figs. 16, 17 and 30).

Fig. 32. Rabbit: twelfth day. Peri-sutural epithelial cuff in longitudinal section with contained suture, resembling a giant comedo surrounded by fibrosis in dermis (× 43).

Fig. 33. Serial section to Fig. 32 showing line of giant abnormal hair follicles formed in secondary spurs which develop radially from the primary peri-sutural epithelial cuff portrayed in Fig. 32. (× 43).

(Figs. 4, 5, 21-24). In sections taken directly through the centre of the point of entry of the suture, only a relatively small wedge of hyperplastic epithelium can be seen invading along the suture line (Figs. 4, 5). This spur is usually better developed on the side of the suture *away from* the incision, i.e. outside the pressure points of the tied sutures (Fig. 37C). If this process is carefully examined in serial sections, it can be seen that a funnel-like sheath of hyperplastic epithelium develops around the suture at the needle puncture site in the epidermis (Fig. 24). Secondary spurs of epithelium are already apparent, extending radially from the primary downgrowth, invading the surrounding, slightly damaged dermis and undergoing internal keratinization (Fig. 24, right).

By the *6th day* (Figs. 9, 10) well-marked zones of epidermal thickening can be seen on either side of the healing incision, seemingly quite unrelated to the original wound. So vigorous are these epidermal reactions to the suture that the hyperplastic and invading epithelium within the incision, frequently—if not usually—unites with a similar and often more massive bar of invading epithelium around the suture (Figs. 10, 16-19, 30, 31). The presence of these thick bars of new epithelium, extending from the suture entry points on either side of the incision and joining with the hyperplastic epithelium in the incision, may alter profoundly the course of the

healing process (Figs. 37C, 37D). These reactions of the epidermis along the suture continue vigorously and are still active on the 15th post-operative day (Figs. 26-36). In all our specimens epithelial response to the suture has usually been much more marked on the outer and deep aspects of the suture than on the inner surface (Figs. 20-36). Only when union (between the regenerated epidermis around the sutures and that in the wound) has been established does the epidermal reaction become well marked (Figs. 10, 34, 35, 37C).

As in the case of epidermis regenerating within the incision, so, too, in epidermis invading along the suture do secondary spurs of varying degrees of thickness and complexity extend from the primary epidermal mass into the surrounding dermis. These secondary spurs develop radially to the primary peri-sutural epithelial mass, like spokes radiating from the hub of a wheel. This process is clearly depicted in Figs. 22, 27 and 28, and especially in Figs. 24, 34 and 35. The secondary spurs undergo internal keratinization, forming cysts within which new and abnormal hair follicles may develop. Patches of cells, constituting the thick stratified epithelial walls of these cysts, may also apparently undergo differentiation into small sebaceous glands. In one specimen the suture actually transfixed a hair follicle which had originally been present in the site of study (Figs. 4, 23). The epithelium surrounding this transfixed hair follicle also became extremely hyperplastic and united with the epidermal bar growing down on the outer side of the suture (Figs. 4, 23).

Once the epidermal spurs (developing within the incision and around the suture) have united, the resulting epithelial bar thickens and undergoes internal keratinization (Fig. 10). As this keratinization extends along the entire length of the bar and reaches the 2 sites of origin, the fragment of tissue lying between the core of the bar and the surface sloughs off (Figs. 18, 19). In consequence of this sloughing process, the entire shape of the surface of the healing wound may alter from a V-shaped dip, described above, to a broad flat groove as portrayed in Figs. 18 and 19. The epithelium now covering the surface of the wound is represented by that portion of the epidermis which remains *below* the central core of internal keratinization. Once more secondary spurs develop from the dermal aspect of this new surface epithelium. With the subsequent internal keratinization of these secondary spurs, new giant, distorted hair follicles and sebaceous glands may develop in animals with-

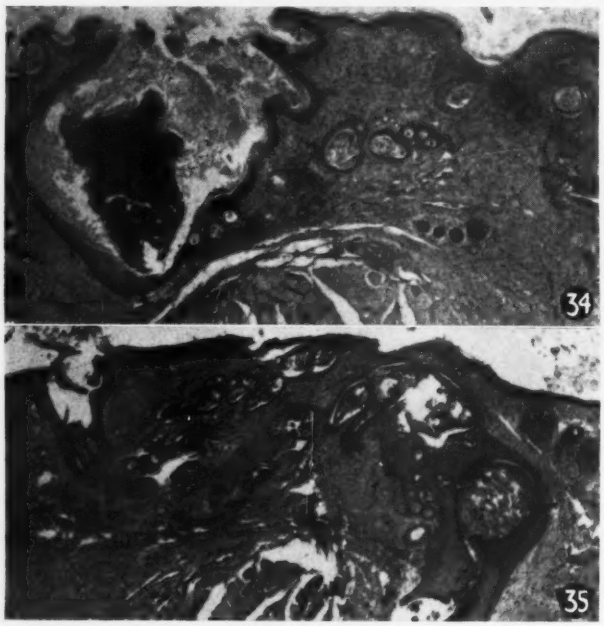


Fig. 34. Rabbit: fifteenth day, showing persistence of massive peri-sutural epidermal invasion and epithelial pearls with giant abnormal hairs developing in spurs which have invaded the dermis radially to the primary peri-sutural epidermal cuff. Incision with related secondary epithelial spurs and pearls, at right of figure. Compare intensity of epidermal reaction at suture and the incision, at this time (× 43).

Fig. 35. Serial section to Fig. 34 showing extent of peri-sutural epidermal reaction and secondary spurs with pearl formation. Compare intensity of reaction to suture with that related to incision, shown at depression in surface epithelium towards right of picture (× 43).

in the remoulded wound surface (Figs. 10-12, 32, 33). On occasion the suture may still be found within a sheath of epidermis, especially near the surface, resulting in microscopic appearances simulating a giant comedo (Fig. 32) such as those encountered in certain cases of malnutrition (Gillman and Gillman[25] Figs. 85-93). An entire row of malformed and incompletely developed hair follicles may be associated with these invasions of epidermis along the sutures (Fig. 33). The invasion of the dermis by epithelial spurs, either in the line of incision or in relation to the suture, seems to be associated with very active fibrosis developing from the infiltrating round cells. That this fibrosis is related in some way to these epithelial spurs is

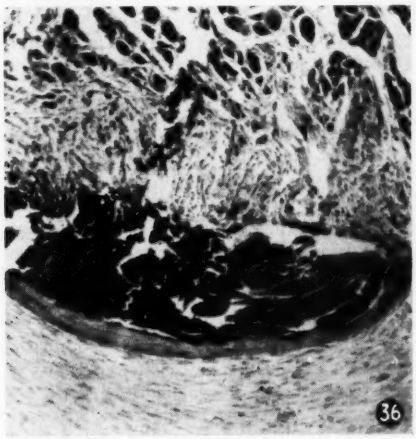


Fig. 36. Rabbit: fifteenth day. High power of portion of suture lying deep in dermis below panniculus carnosus shown in Fig. 20. Stratified epithelium is clearly shown deep to the suture, with the well marked fibroblastic reaction oriented to this epithelium, as compared with the relatively mild fibrosis on the upper, exposed surface of the suture. The fibrosis above the suture is related primarily to the damaged muscle fibres, while that below the suture is organized in relation to the stratified epithelium (× 98). (Compare Fig. 20).

indicated by the fact that in this new, highly cellular fibrous tissue, both the cells and the fibres are orientated parallel to the epithelial line or even tangentially to it. Fibrosis, like round cell infiltration, is always most marked around epithelial spurs and especially around 'pearls'. The rapid increase in fibrous tissue in the dermal portion of a healing, incised wound, which supervenes between the 4th or 5th and the 8th to 10th post-operative days, in rabbits, and between the 6th to 10th post-operative days in Man, seems to be directly related to the intensity of epithelial invasions of the dermis and to the re-modelling of the epidermal surface of the wound at these times.

In Figs. 16, 17, 30 and 31 (3 serial sections of the same wound) the thick epidermal sheath around the suture can be seen uniting with the invading spur within the incision, resulting in the unusual pictures presented. Only from an examination of Fig. 31, and with suitable contrast-staining of keratin and suture (such as can be obtained with Mallory's phosphotungstic acid haematoxylin) can the exact nature be ascertained of the phenomena presented pictographically in Figs. 16, 17, 30, 31 and 37A-37E. From Fig. 31, in particular, it will be seen that the epidermal sheath around the suture was incomplete at one point, and both the keratin (generated on the interior of this epidermal sheath) and the suture evoked a vigorous giant cell foreign body response, clearly evident in preceding serial sections and portrayed in Fig. 30. This type of response simulates very closely that described by Peer[75] by Gordon[36] and by Glücksmann,[32] in relation to skin which has been implanted in the dermis, either experimentally or following trauma. We have not followed beyond the 16th day the fate, in incised wounds, of the epithelium which invades the dermis as deeply as portrayed in Figs. 20 and 36. We can state, however, that there appears to be a much more vigorous and organized connective tissue reaction *below* this epithelium, lying very deep in the dermis, than there is to the upper exposed aspect of the suture (Fig. 36). This further supports our suggestion that in the healing of incised wounds, the epidermis plays an important part in evoking and perhaps even in determining the nature, organization and intensity of the connective tissue response.

Among the complications of the healing process which may result from such epidermal reactions to the suture and to the suture needle puncture wounds, is the supervention of infection within the cyst-like cavities developing within the epithelial cuffs around sutures. This is seen in Fig. 29, where an epithelial-lined cyst containing pus cells is shown. This particular cyst was shown, in serial sections, to communicate with the surface through one tiny orifice, viz. the suture needle entry. However, when such infection spreads to cysts developing in the radially arranged *secondary* spurs around the primary invading bar, multiple epithelial-lined pus pockets may be found deep in the dermis. Such blind pockets, lined by stratified squamous epithelium, were encountered deep in the dermis in serial sections of

the same specimen as that portrayed in Fig. 29.

Similar epithelial-lined cysts have been experimentally produced.[49, 77] Powell White[77] considered these epithelial linings of abscess and oil-cyst cavities, described by him, to be derived by the *chance* contact of the cavities with the epithelium of epidermal appendages. Our own evidence indicates that the surface epidermis, or epithelium of hair follicles, etc. *actively grows* along the tracks formed by suture needle and suture. These are *not passive* implantation cysts of epithelium. Ledingham mentioned that in his material serial sectioning 'usually revealed a point of contact of the new epithelium, and from this point of contact the epithelial covering of the abscess material spreads'. With his view we agree.

We would also like to draw special attention to the marked organizing effect of such aberrant epithelium (especially when sepsis supervenes) on the development of a thick layer of connective tissue. Menkin[64] has drawn particular attention to the production of prolonged epithelial proliferation in subcutaneous tissues, which may follow the severe inflammatory reaction evoked by the experimental injection of pleural inflammatory exudate. Menkin considered that since such epithelial proliferation may simulate certain phases of carcinoma, his findings bore some import for the understanding of carcinogenesis. In our experience epithelial hyperplasia was always very much more marked in epithelial-lined cysts formed about infected sutures. The degree of fibrosis around such cysts was also consistently more advanced than in any other infected portion of the same wound (*cf.* Figs. 27, 29). Our observations thus support Menkin's suggestion that inflammatory exudates vigorously promote connective tissue proliferation over and above the reactions related to the inflammatory process itself. The possible rôle of the lipids in the purulent exudate in provoking this fibrosis merits further study in the light of what has been said above.

As indicated in a previous study[27] cysts in traumatized skin may also arise from obstructed sweat and sebaceous glands and from internally keratinized epithelial spurs. These small cysts may enlarge and frequently become visible macroscopically as yellow sebum- or keratin-containing (*not* pustular) vesicles (milia) in close relation to the wound. Such small sebaceous or keratin-containing cysts which rupture easily, recur intermittently for many years after the injury (up to 30 and more in our own experience) indicating that appendages originally present in the traumatized area, and probably also remnants of the invading epithelial spurs and their derivatives, lie in smouldering activity at the wound site for extremely long periods after the more obvious reactions to the trauma have subsided.

DISCUSSION

Currently maintained views on the healing of incised wounds are summarized diagrammatically in Figs. 36A-36D, where the relative rôles of epithelium, dermal connective tissue and subcutaneous fat are represented. The findings recorded above have been summarized in Figs. 37A-37E. Particular emphasis has been given to the micro-anatomic features of the early phases of repair, the subsequent dominant rôles in the healing process of the epithelium (in the incision and around the suture injuries) and of the subcutaneous fat. The virtual inertness of the stratum reticularis of the dermis, throughout healing, and the punctate suture needle wound scars are also represented. The illustrative diagrams are explained in their accompanying legends.

Key to symbols used in Figs. 36 and 37


=New Blood Clot.


=Emigrated Leucocytes.


=New Capillaries.


=Original Fibrocytes.

=Mobilized Fibroblasts.


=Original Dermal Collagen.


=Scab.

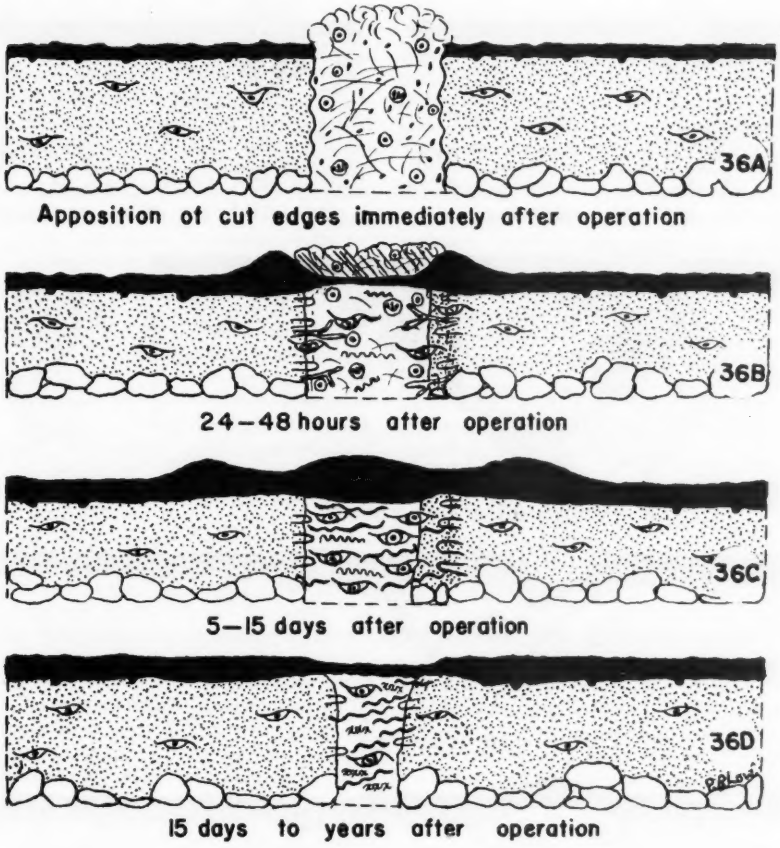

=Reticulin Fibres.


=New Collagen Fibres.


=New Elastic Fibres.


=Epithelial Pearls.


=Giant Cells.



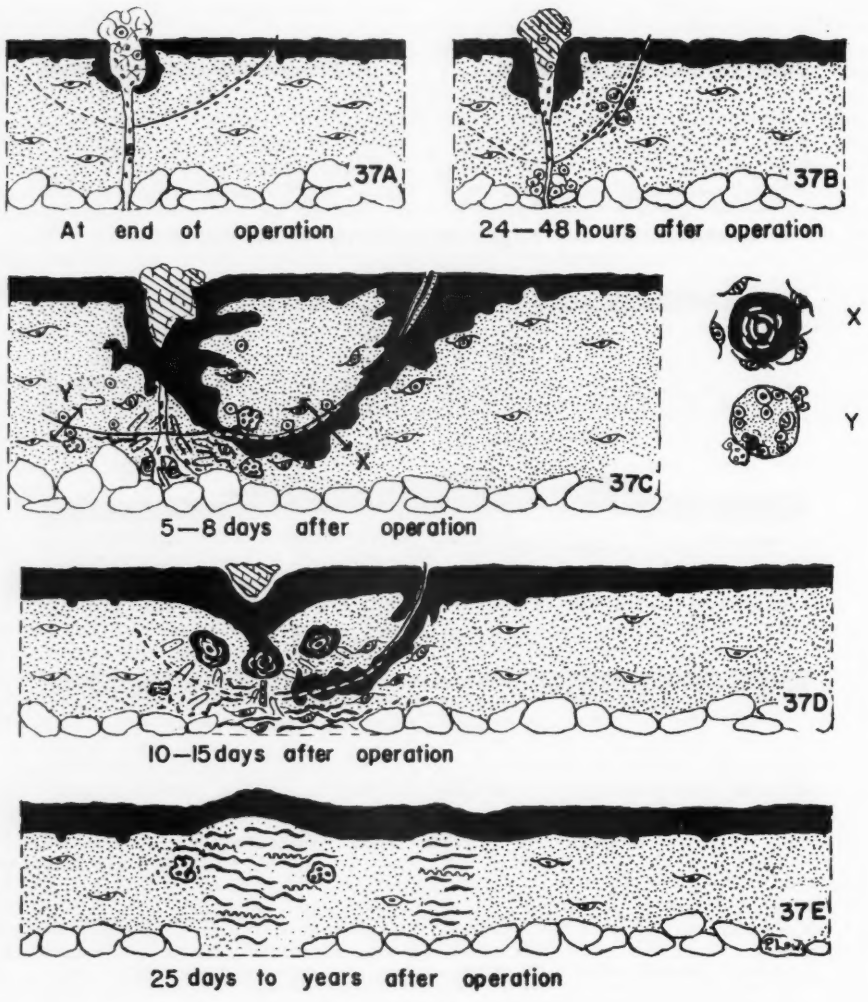
Figs. 36A—36D depict diagrammatically the generally accepted views about the healing of incised wounds.

Fig. 36*A*. Idealized version of the appearance of a surgical incision immediately after closure. The incision (here represented very much wider than reality) is alleged to be filled with clotted blood and/or tissue exudate containing extravasated white blood cells.

Fig. 36*B*. Twenty-four to 48 hours after infliction, the connective tissue of the transected dermis is reported as generating new fibroblasts and round cells. Reticulin fibres are supposed to be numerous at this time. The direction of new vascular growth, i.e. *across* the wound, is emphasized. Epithelial regeneration at this time is considered to be very slight, although the epidermis at the wound edges is hyperplastic.

Fig. 36*C*. Five to 15 days post-operatively, healing is considered to be completed. Fibroblasts may still be rather numerous, collagen fibre deposition well advanced, while vascularity is diminishing. The new epithelium may be hyperplastic, while that near the incision edges has returned to normal.

Fig. 36*D*. After the fifteenth day vascularity diminishes while collagenization increases progressively, resulting in contracture and pallor with associated epithelial thinning. New elastic fibres are considered to be deposited between the third and fifth week. No mention is made by any author, to our knowledge, of the epidermal reactions to suturing or to the histogenesis of the punctate suture wound scars.



Figs. 37A—37E summarize the findings detailed in this report.

Fig. 37*A*. Appearance of incision shortly after operation, showing invariably *inverted* cut edges of epithelium, thin line of fibrinous exudate in incision with small surface blood clot and line of the suture from surface needle puncture and through dermis, with resulting slight peri-sutural haemorrhage.

Fig. 37*B*. Twenty-four to 48 hours after operation, the inverted epithelial edges are thickened and show numerous mitotic figures, the epithelium has *grown* slightly down in contact with the cut surfaces of the dermis, the neighbouring untraumatized epidermis has thickened slightly and peri-sutural round cell infiltration is distinct. The alterations in staining reaction of the traumatized collagen are indicated, as are also the epithelial thickening around the suture needle puncture wounds and the round cell infiltration of the subcutaneous fat. (In this, and in the following diagrams, the reactions around the suture are represented *only on the right*, for simplicity).

Fig. 37*C*. Between the fifth and eighth post-operative days, the epithelial hyperplasia and secondary 'invasive' spur formation are well marked, both in the incision and around the suture. The peri-sutural epithelial cuff may even join with the hyperplastic epithelium in the wound. At this time, fibrosis is well advanced in the subcutaneous fat and in direct relation to the invasive epithelium. The transected dermis is inert.

We now propose to consider some of the implications of our findings for present-day surgical treatment of incised wounds and to indicate, briefly, possible relations between epidermal reactions during the repair process and the pathogenesis of skin carcinomata—particularly those arising in relation to healed wounds.

The most striking phenomenon recorded in this study is the active invasion of the underlying dermis by epithelium in several loci related to the operative procedure. Such invasions during the repair of incised wounds have not been fully described before, although they have been recorded by other workers, apparently unnoticed, in the photomicrographs accompanying their publications. To our knowledge, the only investigator who has specifically mentioned such epidermal invasion of the connective tissues is Bishop (1945) who has meticulously described this phenomenon during the healing of the skin wounds following *removal* of a portion of skin, i.e. in 'excised' wounds.

In recent years the most graphic published pictures of such invasions of the dermis by the regenerating epidermis have been those in the paper of Baxter, Schiller and Whiteside.[6] In particular, their Figs. 1-7 demonstrate clearly the occurrence of the same epithelial reactions in the line of incision as we have described above. These authors have drawn attention to this occurrence in only one of their photomicrographs (their Fig. 2) and in this instance they regard the aberrant epithelium, lying deep in the dermis within the healing incision, 'as proliferating from a hair follicle'. It is difficult, of course, to be sure solely from their published picture that this was *not* in fact the case in this particular instance. However, the general features of the epithelial growth so closely resemble the pictures presented here as to prompt one to ask for proof, from the examination of serial sections, that the deep underlying epidermis in their Fig. 2 was really derived from a proliferating hair follicle and not from the invading epidermis. In the case of their Figs. 3 and 4, and particularly their Fig. 6, the epithelial invasion at the line of incision, although extremely well developed, is not commented on at all by Baxter *et al.* Their Figs. 5 and 7 show internal keratinization with the formation of comedo-like keratinized plugs, almost identical with those portrayed in Figs. 2, 9, 16, 30 and 32 of the present study. This oversight on the part of Baxter *et al.* in failing to draw attention to this interesting phenomenon is probably due to the fact that they did not study serial sections of their material. Had they done so, the genesis of these reactions would no doubt have become as apparent to them as it has to us.

Similar invasions of the dermis by regenerating epithelium in various types of wounds have been recorded pictographically in many pathology books and, apart from Bishop,[10] authors have usually attributed their occurrence to, and even regarded them as evidence of, delay in the healing process (e.g. MacCallum[55]). Percival, Drennan and Dodds[76] also portrayed similar invasions of the dermis by the regenerating epithelium during repair, either of contaminated wounds (their Figs. 148, 149) or of varicose ulcers (their Figs. 151, 152). Again, very little attention is drawn to these epithelial invasions which are regarded by the latter authors as an aspect of the *pathology* of the repair process in the skin. The present investigation reveals, beyond doubt, that *such invasions of the dermis by regenerating epithelium are a consistent phenomenon in uncomplicated repair of cutaneous wounds.* However, this normally occurring epithelial invasion of the dermis may simulate (and perhaps, in special circumstances, even contribute towards the precipitation of) cutaneous neoplasms, in a manner which will be discussed briefly below.

For the moment, it may be of value to consider the implications of the above findings for our orientation towards the usual present-day surgical techniques of dealing with cutaneous injuries.

Although the greatest care was taken, both in human and in animal operations, to appose the cut edges of the incision as accurately as

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Fig. 37*D*. By the tenth to fifteenth days, the fibrotic reaction and associated vascularity in the subcutaneous fat is well advanced and new capillaries are now growing surface-wards into the gap between the 2 edges of the transected dermis. The direction of new vascular growth, as found by us, should be compared with the generally maintained views diagrammatized in schemata 2 to 3 above. The epithelial regeneration seems to have abated or to have been brought under control by the vigorous peri-epithelial round cell and fibrotic reactions. Numerous internally keratinizing epithelial pearls represent the remnants of the 'invading' epithelial spurs shown in Fig. 37 C.

Fig. 37*E*. From the twenty-fifth post-operative day onwards, vigorous epithelial and connective tissue reactions, initiated by the injury, abate somewhat. Collagen deposition is well marked, although new elastic fibres may not appear for years. The epithelium slowly thins to assume scar-like characters.

possible, the epithelial edges were, nonetheless, always found initially to turn and subsequently actively to grow downwards into the dermis (Figs. 37A-37C). This was observed even within the first 24 hours after making the incision as well as in all later specimens. We have yet to see a low-power photomicrograph of good sections of healing incised wounds in which such inversion of the cut epidermal edges is *not* depicted.[21, 67] Even a careful naked-eye examination of an uncomplicated healing incised surgical wound, at about the 5th to 8th post-operative day, will consistently reveal the line of the incision to be *inverted*, after removal of the blood crust or 'scab'. This inversion of the cut edges and actual active ingrowth of regenerating epithelium can be demonstrated consistently in good microscopic sections of healing incised wounds, up to 25-30 days post-operatively.

This infolding of the epithelium may be an *active* response to some trophic stimulus from the dermis; or it may well be the result of some peculiarity of epithelial growth. In a previous report[27] we drew attention to Medawar's findings[62] in tissue culture, substantiating our view that the cells at the edges of an interrupted epithelium will continue to grow until they come into contact with other epithelial cells of their own type. Glücksmann's fine illustrations[32] of cyst formation by subcutaneously implanted autografts of skin in rats, and our own unpublished confirmation of his views also support this opinion. Findings recorded by Powell White[77] and later by Ledingham[49] also substantiate this observation and opinion. In the light of all this basic information concerning the pattern of epithelial growth under various experimental conditions, our observation that the epidermis in an incised wound always folds inwards, even when everting sutures are used, is not surprising. Considerable experimental work is still required to determine the method of apposing the edges of an incised surgical wound which will ensure the most rapid healing, the best scar and the fewest complications within the scar.

That the invasions of epithelium along the needle puncture wounds is a constant phenomenon, occurring in almost all surgically sutured human wounds, is undeniably substantiated by the regular occurrence in all human and animal material of punctate circular scars symmetrically arranged on either side of the incision. These punctate suture scars have not, to our knowledge, been given any attention by previous investigators. In surgical practice on human subjects, it is usual to remove the sutures between the 6th and 10th post-operative days. Nevertheless, the punctate scars at the suture sites persist for months and even for years. It is perhaps important to realize that such punctate scars manifest all the histological features of incisional scars and yet, according to our clinical observations in Man, these scars can and usually do ultimately disappear. Why these scars disappear, whereas incisional scars do not, remains a problem meriting further investigation. In how far the removal of sutures between the 6th and 10th post-operative days and/or the type of suture used in wound closure, facilitates the development and subsequent resolution of such suture scars, also seems worthy of more intensive study.

It is widely recognized that several complications may supervene in scars. For example, in persons with a tendency towards keloids, such as Negroes, both incisional scars and the multiple suture needle puncture wound scars may become keloidal. The intermittent recurrence of discharging, non-septic, small, sebaceous-like cysts within the incisional scar, as well as in the related suture scars, also occurs quite frequently in general surgical practice. The histological basis for these latter reactions is now clearly apparent from the present report.

Bearing in mind the histogenesis of several types of scars developing in relation to the repair of incisions, as well as the complications which may occur in relation to such scars, the time may well have come for re-assessing the techniques generally adopted for suturing the skin. It may be that despite the laboriousness of the procedure, subcuticular stitching should be more widely used, in order to avoid any damage to the epidermis, other than that which is necessary in making the incision. Perhaps, too, the simple technique for wound closure, using plaster strips, recommended some years ago,[79] or some more convenient modification, may also merit consideration for wider adoption in surgical procedures. A combination of this latter method with a limited number of tension sutures may contribute much towards more satisfactory repair of wounds and may even diminish the number of hypertrophic scars, keloids and other complications in scars.

Apart from benign complications in different types of scars, attention should also be directed towards the possible significance of the findings recorded here to the well-known relationship between cutaneous neo-

plasms and injury to the skin. Ewing[20] has reviewed much of the clinical and experimental data on the association of epidermal carcinomata with injury and especially with scars, while Goldhahn[33] has reported the specific localization of metastatic carcinoma within suture needle puncture sites following radical mastectomies for primary carcinoma of the breast. This may have been due to local implantations in the skin of the malignant breast cells during suturing. However, the apparently regular, if not completely consistent, experimental precipitation of neoplasms in sites of injury by the applications of carcinogens to scars is not so easily explained. Deelman's original findings[18] have been confirmed by many subsequent investigators.[22, 56, 72, 78]

The literature reflects considerable controversy concerning the relative rôles of the dermal and epidermal changes preceding and associated with the pathogenesis of spontaneously occurring (or experimentally induced) skin cancers in scars.[7, 47, 51, 60, 72, 93] Since a carcinoma is an *epidermal* neoplasm, attention has been devoted primarily to an analysis of the factors regulating *epithelial* regeneration. However, several of the abovementioned authors incline to the view that changes in connective tissue may be primarily responsible for the initiation of skin tumours. Menkin (*loc. cit.*) also suggests that chronic inflammatory *dermal* changes may play a part. During the healing of a skin wound, both epithelium and connective tissues are activated to regenerate and one may justifiably regard a healing wound as a regulated or controlled 'new growth' which is arrested by unknown mechanisms on the 'completion' of the repair process. Our studies of healing wounds clearly demonstrate that the epithelium begins to regenerate *first*, and long before there are any easily detectable signs of connective tissue regeneration. In fact, it would seem that in healthy healing the rapidly growing epidermis plays a prominent part in *initiating* the regeneration of the underlying connective tissues in the dermis. On the other hand, during the later stages of repair the connective tissues seem to control, if not inhibit, unrestricted epithelial regeneration. There is some support for this possibility from our own studies, as well as from the reports of several other investigators.

Recently it has been suggested[9] that the precipitation, by injury, of carcinomata of the skin of animals pre-treated with carcinogens was not determined so much by the effects (of both the carcinogen and trauma) on the epidermis as upon the dermis. This was in conformity with the opinion expressed by Orr on several previous occasions.[72] While this manuscript was in preparation we received the most recent publication by Orr and his co-workers on this aspect of the problem. Marchant and Orr refer to the work of Linell,[51] who reported that superficial trauma, involving the epidermis alone, did not lead to an increased tumour incidence (in carcinogen-treated skin), whereas deeper trauma involving also the connective tissues *was* followed by an increased tumour response *in the region of the scars*. In previously reported experiments Orr[70, 71] had found that:

> 'Insertion of threads (sutures) into the sub-epidermal tissues without operative treatment of the epidermis itself resulted in an accelerated incidence of tumours in mice which were subsequently painted with carcinogens.' In their present experiments Marchant and Orr,[60] however, state: 'Trauma to the sub-epidermal tissues inflicted subsequent to the carcinogenic treatment has not increased the rate of tumour appearance.'

It is relevant to the present analysis to draw attention to Linell's findings, to similar findings by Lacassagne and Latarjet, as well as to Marchant and Orr's report that tumours related to wounds inflicted in methylcholanthrene-treated skin, seem to have a predilection for the scar itself. Marchant and Orr remark on their own findings that there seemed to be a definite

> 'tendency for tumours to arise in the bridge of epithelium over the scar surrounding the graft rather than on the graft itself. The area of the scar surrounding the graft was of the order of one-sixth the area of the graft yet there were as many persistent tumours in scars as on grafts themselves . . . ".

It is important, at this stage, to draw attention to the significant fact that, in the experiments reported by Marchant and Orr, in which they failed to increase the incidence of tumours by traumatizing the dermis through the introduction of cotton threads or of hot wires, neither the threads nor the wires were introduced into the dermis *through the epidermis of the methylcholanthrene-treated area.* Both the hot wires and the cotton threads were inserted through the skin *outside* the carcinogen-treated area and drawn out *beyond* this same area.

Apart from the work of Paletta *et al.*,[73] repair of incised wounds in carcinogen-treated skin of animals, and the relations of this healing process to the pathogenesis of methylcholanthrene-induced carcinomata in the sites of injury, have not been studied systematically. In our previous analysis of the healing of skin graft donor sites and of the histogenesis of the 'taking' and shedding of various types of

grafts, we constantly found vigorous epithelial invasions of the new connective tissue with subsequent epithelial pearl and foreign body giant cell formation. We have now also established these latter reactions to be regular and normal concomitants of repair of *incised* wounds as well as of suture needle puncture wounds. We have demonstrated, furthermore, that invasion of dermal tissues by regenerating epidermis is a consistently normal event during healing. This invasion of the connective tissues always occurs at sites of interruption in the continuity of both epidermis and dermis, where there is an associated initial *depression* of connective tissue regeneration. Moreover, such epidermal invasions seem to be attributable not only to a break in epithelial continuity, but to associated changes in the organization, chemical constitution and regenerative capacities of the subepidermal collagen.

We have been especially impressed by the constant association (with epidermal invasions) of alterations in the staining reactions of dermal collagen such as those mentioned above and portrayed in Figs. 4, 5, 8, 11, 21 and 26. We have already described,[28-30] and will soon do so in greater detail, the staining reactions of a 'fourth' type of fibre in the dermis and other connective tissues, i.e. in addition to collagen, elastic and reticulin fibres. Changes in the tinctorial character of collagen, leading to affinity with basic and elastic tissue stains, such as occur following mechanical, radiation and possibly metabolic traumata, seem to carry considerable significance for epithelial regeneration. Orr[72] actually records his impression that the earliest reactions induced by carcinogens seemed to occur in relation to intra-dermal 'scar-like' areas showing 'excessive amounts of elastic tissue'. There have also been several recent studies of the 'increase in elastic tissue' which supervenes in the dermis of methylcholanthrene-treated mice prior to the onset of carcinoma.[54] Vernoni[93] reported fully on similar increases in 'elastic tissue' in association with epidermal carcinomas in Man and he concluded that:

'Skin cancerisation in man and in animals is primarily connected with changes in the dermal connective tissue and secondarily with changes in the epidermal tissue . . . In fact, it is more probable that the slow changes of collagen fibres, of elastic fibres and of fibroblasts must liberate breakdown products favourable to cell multiplication.'

The alterations in the staining reactions of collagen in grafts, in traumatized dermis related to incised, puncture and excised wounds, as well as to carcinomas supervening in human skins, caused by chronic roentgen ray and/or chronic ultraviolet irradiation dermatitis, have received special attention by our group. Suffice it to say here that proliferating epidermis, whenever it invades the underlying dermis, seems to do so in regular relation to profoundly altered collagen,[28-30] usually a form of 'elastotically degenerated' collagen.

It is of interest in this regard to draw attention to work[87, 88] quoted by Vernoni (*loc. cit.*) that:

'Other experimental facts . . . support the concept that fibrous elastic tissues such as the dermis, contain and can set free substances capable of promoting tissue growth *in vitro* . . . This work has shown that the pituitary and the fibrous *elastic tissue* of the aortic wall possess the greatest growth-promoting power.'

It is our impression that the formation of epithelial pseudo-pegs and pearl-like structures and the connective tissue reactions thereto, supervening during the repair of various types of cutaneous injuries, are not always easily distinguishable from the almost identical pre-cancerous reactions in the skin of Man. It may well be, in the healing of wounds and in pre-cancerous states, that the epidermal proliferations, and especially the invasions of the dermis by the epithelium, arise in response to alterations in the underlying collagen, operating on an epidermis sensitized by interruption in its continuity. It seems possible, too, that spurs of epidermis which actively invade the dermis may become the foci for malignant transformations in the epithelium. This seems very likely in view of the now well-established fact that the epithelium related to scars is particularly predisposed to malignancy. The invasive behaviour and the prolonged reactions in the epidermis which we have shown to be a constant feature of healed wounds, would also explain the maintained sensitivity to carcinogens of epithelium in the immediate vicinity of scars.

We are fully aware that carcinomatosis is a *rare* complication of scars which develop in clean surgical incisions in man. However, there is a body of almost irrefutable evidence,[20, 51, 64, 77] reviewed in detail by many investigators, that chronic inflammation in parenchymatous organs, chronic ulcers of viscera (such as the stomach and the cervix uteri) and even of the skin, may be and frequently are followed by the development of malignant neoplasms. In attempting to relate our findings in healing wounds to carcino-

genesis, we are motivated by the belief that any circumstance in which there is tissue growth, and especially regeneration, may provide information of ultimate value in understanding more about the pathogenesis of malignant growth.

SUMMARY AND CONCLUSIONS

1. The histogenosis of repair in experimentally inflicted incised wounds has been studied in healthy human and animal subjects.

2. The need was shown for *serially* sectioning material in histological studies of the dynamic 3-dimensional changes in healing wounds.

3. Particular emphasis was laid upon the following *epidermal* reactions during the primary repair of incised wounds:

(a) The epithelium, at the opposed cut edges, consistently inverts towards the dermis, irrespective of the precision of suturing on closure.

(b) Mitotic figures are common in the zone of injury at an early post-operative date. The regenerating epithelium covers the incision within 1 to 2 days, by growing *beneath* the clot in the wound and in direct contact with the intact underlying dermal fibres.

(c) This coverage of the wound by the regenerating epidermis is the *first* irrefutable sign of repair and occurs long before any evidence of connective tissue regeneration in the line of injury. Epidermis grows down to and along the relatively inert cut edges of the dermis.

(d) Spurs of regenerating epidermis regularly invade the dermis, at and near the site of injury, and develop into internally keratinizing cysts, which may, or may not open to the surface and give rise to neogenesis of abnormal hair follicles, associated sebaceous glands, and sebum and/or keratin containing cysts, immediately related to the scar.

(e) These epithelial reactions in the line of incision are far from completed by the 15th post-operative day.

4. It has been clearly established that epidermal reactions identical with those summarized above, invariably supervene in relation to the suture needle puncture wounds in the epidermis and to the suture itself. These reactions are usually as vigorous as, and often more so, than those in the incised wound itself.

5. It was also shown that the regenerating epithelium, related to the suture, may extend well into the dermis and persist there—as epithelial pearls and/or cysts—at least for weeks, and perhaps for longer. Moreover, these epidermal invasions, related to the suture, may join with those in the incision, thereby profoundly influencing the ultimate pattern of scar formation at the incision.

6. Observations concerning the dermis, at the incision and related to the skin sutures, include:

(a) Early alterations in the morphology and staining reactions of the collagen fibres.

(b) An apparent dependence of fibrosis, in the *dermis* (not in the subcutaneous fat or muscle), on the round cell infiltrations evoked by epidermal invasions.

(c) The primary sites of connective tissue regeneration, i.e. the subcutaneous fat and muscle. Later, increasing epidermal invasions and the related round cell infiltrations, lead to fibrosis in the immediate subepidermal tissue.

(d) Fibroblasts, which are responsible for connective tissue regeneration, and seem to be derived primarily from haematogenous lymphocytes and monocytes which infiltrate first the injured fat and muscle, and later the subepidermal tissues.

(e) The cut stratum reticularis of the dermis, which remains inert almost throughout the early phases of repair, and is the last portion of the incision to heal.

7. The implications of the presently recorded findings are reviewed and a plea made for a re-assessment of the suturing methods presently used in modern surgery, for the closing of skin wounds.

8. Particular attention is devoted to an analysis of the possible significance of the foregoing findings for the genesis of skin carcinomata, keloids, sebaceous cysts and other complications in healed skin wounds.

9. It is suggested that alterations in the dermal fibres, induced by various types of physical, chemical and metabolic traumata, may perhaps play a most important rôle in initiating and determining the pattern of epidermal responses to such injuries.

10. There seem to be many similarities between the histological appearances of epidermal and dermal reactions to incised wounds and to sutures, and those which have been observed during the early stages in the pathogenesis of skin carcinomata in Man and in experimental animals.

II. THE HEALING OF WOUNDS INVOLVING LOSS OF THE SUPERFICIAL PORTIONS OF THE SKIN

The nature of healing in the skin (and the factors affecting this process) have been studied closely for the past 30-40 years, mainly to expedite the normal healing process and to eliminate or minimize the formation of scars which may be unsightly or may result in severe contracture with consequent deformity or limitation of function.

On the basis of the *ultimate* pattern of healing, repair processes in the skin may broadly be classified into 3 major categories:

1. Healing with *complete* restitution of the original morphology of the skin, e.g. following superficial abrasions or burns.
2. Healing with *partial* restitution of the original morphology of the skin ('scar-like'), e.g. after the healing of thin, split-skin, graft donor sites.
3. Healing with scar formation and with the almost total loss of the original structure of the skin, e.g. following deep incisions or deep burns, etc.

The study of the healing of burns of varying depths has indicated quite clearly that, in the absence of local or systemic complications, the *depth* and *not* the *extent* of the lesion determines the outcome of the *local* healing process. As indicated in earlier papers,[28-30] this applies also to the healing of *incised* wounds, i.e. where there is no *loss* of tissue.

Many reasons have been given for the presence or absence of a scar, according to the depth of the healed lesion. The question is: Wherein lies the remarkable capacity of the skin to reconstitute itself completely and without scar formation? *Both* of the component tissues of the skin, epidermis and dermis, are capable of considerable regeneration. Consequently scar formation in healed wounds has been attributed variously to incomplete or *altered* regeneration either of the epithelium or of the connective tissue. Thus, Bishop[10] maintains that the regenerative capacity of the epithelium is directly determined by the rate and nature of regrowth of the connective tissues. On the other hand, according to most authors, the generally adopted practice of treating deep burns by skin grafts, to prevent scar formation, is based upon the view that, in deep lesions, glandular sources of epithelium lying deep in the dermis are destroyed.[15] The avowed objective in treating wounds with skin grafts is to provide a source of the missing tissue, viz. epithelium. That this latter view is *not* entirely in conformity with available facts was clearly demonstrated by Billingham and Reynolds (1952), who failed to prevent scar formation and wound contracture in experimental animals even though they provided an ample source of viable epithelium. These authors were forced to conclude that:

'It may therefore be concluded that the provision of epithelial coverage on an area of full-thickness skin loss will not prevent wound contracture; the presence of at least some dermis is necessary to do this.'

A new approach therefore seems necessary for understanding the factors determining the nature of the healing process, if an unassailable rationale for skin grafting is to account for the empirically well-established success of this procedure in avoiding serious scarring.

In a previous publication[27] attention was drawn to the alterations in the healing of a particular type of cutaneous wound, following the application to the injured area, separately, of grafts of *each* of the 2 major components of the skin. Several observations were recorded which, while explained at that time on accepted data about wound healing, were nevertheless puzzling. Among these findings was the frequent occurrence, both macroscopically and microscopically, of numerous small cysts (milia) in the dermis. These cysts arose from 2 main sources:

(1) From obstructed sweat and sebaceous gland ducts.

(2) Less significantly, from internally keratinizing epithelial cysts, derived from *surface* epithelium which had apparently invaded the

underlying connective tissues at one or other stage during the healing process.

At the time of this publication we accepted the generally held view that granulations form in the blood clot which invariably covers a thin split-skin (Thiersch) graft donor site. On closer study it became apparent that these cysts were most common in graft donor sites to which various types of grafts had been applied. We therefore attributed these cysts to obstruction, by the grafts, of the growth of the epithelium from the skin appendages. The rarity of cysts in ungrafted donor sites therefore puzzled us because if (as generally stated) granulations or connective tissue regeneration is a necessary *precursor* for epithelial growth, *how does epithelium from skin-gland ducts and hair follicles grow through the layer of granulations, alleged to form at the onset of healing in the surface blood clot or the serum expressed therefrom?*

There could be no doubt, from our own material in particular, but also from the careful auto-studies by Bishop, that new connective tissue always develops in sites of skin loss involving dermis, however superficial these lesions may be. We had constantly encountered, at the tenth post-operative day, a thick layer of what we then called 'granulations', underlying the new epidermis which had covered the raw area.

It seemed worth examining more closely the *early* stages in the healing of split-skin graft donor sites. Besides, our earlier observations were very much at variance with widely held views that healing of thin split-skin graft donor sites is 'completed' by the ninth or tenth post-operative day. During the present investigation many other fascinating, if not fundamental, questions were formulated. Among these were:

Does the skin in a Thiersch graft donor site ever reconstitute itself completely?

How does the new epidermis covering a spontaneously healing graft donor site become so firmly attached to the newly regenerated upper layers of the dermis?

What are the relative roles of epidermis and dermis in regulating the regeneration and subsequent cessation of tissue growth?

What are the sources of the new epithelium and connective tissue in the healed donor site?

A meticulous examination of *all* stages in the healing of a graft donor site, and particularly of the earliest phases, would probably illuminate the healing process generally and might provide further understanding of the connective tissue-epithelial relations during repair of other types of skin lesions. There was also the possibility that, as a healing wound (at certain stages and in certain ways) might be regarded as a 'new growth' of tissue, an understanding of the carefully regulated growth characteristic of healing might divulge some of the disturbances in connective tissue-epithelial relations which permit the unrestricted autonomous growth of true neoplasms.

MATERIALS AND METHODS

The surgical and histological procedures in this study were identical with those previously detailed.[27] Thiersch grafts about 6-7 inches long by 4 inches wide were removed with a Blair knife from the antero-medial aspect of the arms of 13 human volunteers. In one of our experimental subjects all the operative procedures, including the taking of numerous biopsies, were done under hypnosis.

The Thiersch graft was treated in various ways to provide separate epidermal and dermal grafts for re-application to certain portions of the donor site, but these procedures will not be detailed in this communication. We need state only that after removal of the graft, biopsies were taken at the edge of the donor site, thus *including both* the healing wound and (as a precise control for each patient at each biopsy) the neighbouring untraumatized skin. In all cases biopsies were $\frac{1}{2}$-$\frac{3}{4}$ inch long and of considerable depth. Biopsies of the untreated donor site were taken at a distance from the grafted areas, and also from the sites of any previous biopsies. All specimens were gently stretched out on cardboard to keep them straight for fixation and processing and to facilitate embedding.

In this study, however, a much greater amount of material was available than in the previous one, due to the kind assistance of several new volunteers. In particular, we wish to thank, once more, Mr. H. W. F. Bannell who very kindly provided us with repeated biopsies from the first post-operative day until the forty-eighth.

We were particularly concerned, in the present study, to obtain specimens from donor sites *during the first few post-operative days.* The earliest specimen available in our previous analysis was at the seventh post-operative day. The present study provided an excellent opportunity of studying the earliest phases in the healing processes from the first 24 hours post-operatively.

Once some of the important differences became apparent between our findings and those generally accepted in the literature, we

made particular efforts to obtain, as additional controls, *early* specimens from donor sites to which *no dressings had been applied* during the first few post-operative days. In 2 volunteers, therefore, we covered the wound with a well-padded vaccination guard which did not come into contact with the wound at all, but rested lightly on the surrounding untraumatized skin only. We then applied a bandage over the top of the guard to protect the wound from infection or any other trauma. In these 2 cases it was therefore possible to obtain biopsies from *totally* undamaged healing wounds which had not been tampered with or traumatized in any way post-operatively.

All the biopsies from the 13 healthy human volunteers studied (8 Europeans and 5 non-Europeans) as well as when they were taken are tabulated in Table I. The biopsies from untreated split-skin graft donor sites, previously described, were carefully re-examined and included in the material tabulated.

TABLE 1: SUMMARY OF BIOPSY MATERIAL FROM 8 EUROPEAN AND 5 NON-EUROPEAN HEALTHY HUMAN THIERSCH GRAFT DONOR SITES.

Post-Operative Time		*Number of Specimens*
Days:	0	10
	1	4
	2	3
	3	1
	4	4
	5	1
	6—10	10
	14—20	19
	21—30	8
	67—80	7
Years:	2	1
	8	1
	Total	69

The histological procedures were again the meticulous *serial section* methods on formalin-fixed, wax-embedded material detailed before.[27-30] A far greater number of staining methods was used in this study than in the previous one. In particular, Mallory's phosphotungstic acid haematoxylin was found unsurpassable for detailed epidermal morphology. The periodic-acid Schiff method[50] provided excellent demonstration of carbohydrate, while the Wilder reticulum technique and a modified Masson method, using Ponceau-2 R and light green, were found to surpass the standard Mallory's triple method for displaying connective tissue detail as well as cytology of both epithelium and connective tissue.

Photographic technique, including methods of estimating magnification, have been detailed.[28-30]

OBSERVATIONS

GENERAL FEATURES OF THE HEALING OF THIERSCH GRAFT DONOR SITES

Although the following descriptions are based upon an unusually large number of specimens derived from human volunteers, not all the specimens at our disposal will be described. Most of the photomicrographs presented have been taken from a series of specimens derived from a single subject (Mr. H. W. F. Bannell), who very kindly permitted an extremely large number of experimental operations. However, information gleaned from the study of other specimens available to us will be presented to clarify or amplify descriptions. Moreover, in several cases specimens from other subjects displayed the phenomena under discussion more clearly and were used for illustrations. Consequently the description of photomicrographs is illustrative of the main phenomena, seen in all our specimens, during the healing of wounds resulting from the loss of only the upper layers of the dermis together with the entire epidermis and the uppermost parts of the skin appendages.

Fig. 1 is a low power view of the general structure of the skin of the antero-medial aspect of the arm, the area used most frequently in the experiments. The 'normal' epidermis in this region is only 6-8 layers thick. There is very little keratinization, the basal layers of the epithelium are only low columnar and the upper layers do not show any marked degree of flattening. Prickle cells are very poorly developed in this region, unlike in other areas of the skin, e.g. the sole of the foot and the palm of the hand. The rete pegs (also very poorly developed in this area) occur irregularly, are shallow and very rarely branched. The epidermal folds in the section in Fig. 1 are largely artefacts resulting from contraction of the skin (after excision) and fixation despite previous straightening on cardboard. Because the dermis in this region is so thin, these folds develop more easily than in biopsies removed from other skin fields. The dermis of the skin covering the antero-medial aspect of the fore-arm is constituted of the 2 distinct layers which are also found in other areas. These layers are:

ABBREVIATIONS USED IN REFERRING TO STAINING METHODS IN LEGENDS TO FIGURES

H and E	Haematoxylin and eosin.
PAH	Mallory's phosphotungstic acid haematoxylin.
PAS	Periodic acid Schiff.
Orcein H and E ..	Orcein, haematoxylin and eosin.
EH and E	Weigert's elastic stain, haematoxylin and eosin.

Note: Magnifications are those of the photomicrographs before their reduction, in printing, to about $\frac{7}{8}$ of the original size.

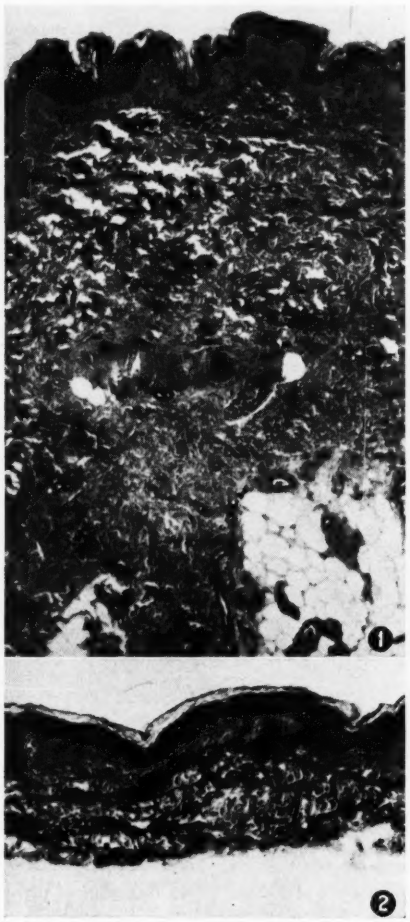


Fig. 1. Histological appearance of the normal skin of the area used in this study, viz. the antero-medial aspect of the arm. The thickness of the epidermis, the poorly developed rete pegs, the striking stratum papillaris of the dermis and the depth of stratum reticularis and the associated skin appendages are well shown. (EH and E. × 43).

Details of normal epidermis are shown in Fig. 37, p. 168.

Fig. 2. Low power view of a Thiersch graft removed from the antero-medial aspect of the arm of one of our subjects. The usual depth to which such grafts were taken is clearly shown to be in the upper portions of the stratum reticularis of the dermis. Again the morphology of the epidermis and of the epidermal-dermal junction for this area are shown. (EH and E. × 43).

1. The *stratum papillaris*, the immediately subepidermal region, constituted of a feltwork of very delicate collagen and elastic fibres, together with a considerable preponderance of reticulin fibres. The loose texture of fibres in this region is in striking contrast to the closer more compactly arranged fibres in the more deeply situated stratum reticularis of the dermis. Moreover, the collagen fibres in the stratum papillaris, seem to be finer in character than those seen in the coarse bundles of the stratum reticularis. The general direction of the fibres in the stratum papillaris, relative to the overlying epidermis, seems to be more consistent than in the stratum reticularis.

2. The *stratum reticularis*. Collagen fibres predominate and are arranged in coarse bundles, which in turn are arranged in various directions as in a lattice.

We have made careful studies of the nature and organization of the fibres found in the dermis of different skin fields covering the human body. This investigation has not only included specimens obtained from many different skin fields but also from the same skin fields in subjects of different ages and sexes and with different degrees of natural pigmentation. We will not give a long description of the basic structure of the skin in different parts of the body, albeit a knowledge of the basic histology of different skin areas is fundamental in the interpretation of the reactions of various skin fields to the same trauma. A full description of the histology of the skin will be provided in other communications.

Apart from the organization of the fibres in the stratum papillaris and the stratum reticularis of the dermis, attention should also be drawn to the fact that there is a relative scarcity of epidermal appendages in the dermis of the area of the skin here studied. Hair follicles are rare, as are also the associated sebaceous glands, although sweat glands are not as uncommon in the antero-medial aspect of the forearm as they are in other regions of the body studied by us. Bishop (1945) has drawn particular attention to the organization of the connective tissue surrounding the hair follicles, sweat glands and sebaceous glands in the skin. Since we have also found that the connective tissue in this peri-follicular zone and

surrounding the various glandular elements of the skin is highly reactive during wound healing, we would also emphasize that this connective tissue is extremely similar to that found in the stratum papillaris of the dermis, as described above.

Fig. 2 illustrates the thickness of tissue removed experimentally, as a thin split-skin (Thiersch) graft. The entire epidermis as well as the full depth of the rete pegs and the mouths of the sweat glands, sebaceous glands and hair follicles were removed as part of the graft. Also, the stratum papillaris and the upper portion of the stratum reticularis constituted part of the resected tissue.

The surface of the wound remaining after the removal of the graft therefore consisted of blood clot derived from the blood shed at the time of the operation. Below this clot lay the raw, denuded stratum reticularis of the dermis. As indicated above, under descriptions of the methods used, in several of our subjects dressings were *not* applied to the denuded dermis during the first few post-

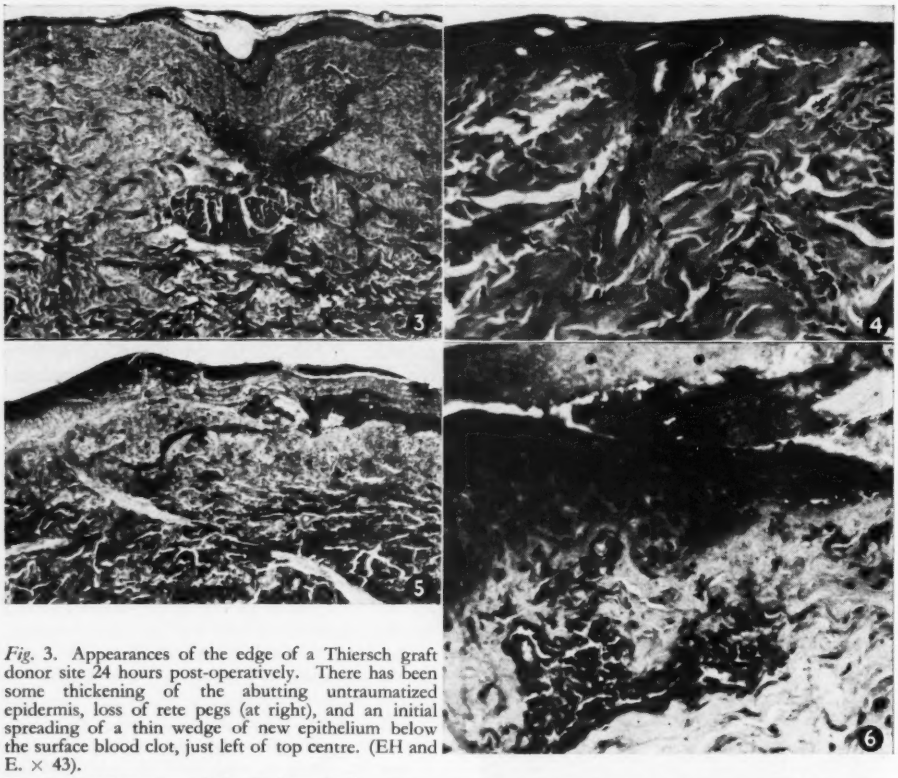


Fig. 3. Appearances of the edge of a Thiersch graft donor site 24 hours post-operatively. There has been some thickening of the abutting untraumatized epidermis, loss of rete pegs (at right), and an initial spreading of a thin wedge of new epithelium below the surface blood clot, just left of top centre. (EH and E. × 43).

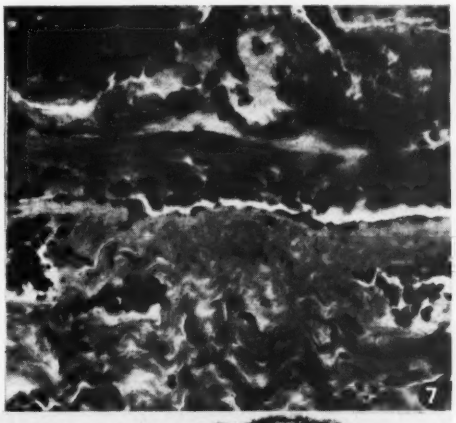
Fig. 4. Another view of the 24 hour healing donor site taken from the same specimen as Fig. 3 to show the appearance of a sweat gland duct in the middle of the denuded area. It can be seen, even at this low power, that the epithelium of the sweat gland duct is regenerating. Confirmation of this opinion is provided in Fig. 35, a high power view of this same sweat gland. Initial peri-vascular cuffing with round cells is already apparent in the dermis. (EH and E. × 112).

Fig. 5. Junction of untraumatized epidermis (at left) with donor site at 2 days. The thickening of the untraumatized epithelium is now more apparent, as is also the thin wedge of epidermis (top centre) migrating below the blood clot (at right). The collagen lying deep to traumatized portion of the dermis shows an alteration in staining reaction with an increase in the basophilia of the collagen, with the present stain. (PAH. × 43).

Fig. 6. Higher power of edge of donor site portrayed (at left) in Fig. 5 to show the manner in which the upper layers of the stratum spinosum of the epidermis migrate across the denuded donor site. These migrating cells can be seen to be darker staining than the deeper cells, this increased 'blackness' being due to the presence of glycogen in cytoplasm. The stratum granulosum has disappeared in this region. (PAS. × 200).

operative days. In these cases bleeding was more profuse, and since oozing persisted for 6-8 hours post-operatively, the clot was thick, firm and crust-like 24 hours after the operation. It will be seen from Figs. 2 and 3 that no epidermis or surface epithelium remained in the site of operation. This was constantly confirmed in serial sections of biopsy specimens of the donor areas.

Figs. 3 and 4 show the appearances of wounds produced in our experimental subjects, *24 hours post-operatively*. It can be seen that even 24 hours after the operation there is some thickening of the untraumatized epidermis at the margin of the wound. Moreover, there is evidence of initial hyperplasia of the epithelium lining the hair follicles and the ducts of sweat glands. This is clearly evident in Fig. 4 and more obvious in Fig. 35, a high power view of the sweat gland portrayed in Fig. 4. Despite the trauma occasioned by the initial operation as well as by the surgical interference in obtaining biopsy specimens, there is very little oedema in the surface layers of the traumatized dermis. In Figs. 3 and 4 a thin layer of blood clot remaining, after the removal of the *tulle gras* dressing in this case, can still be seen overlying the denuded stratum reticularis of the dermis (see left of Fig. 3). A thin wedge of epidermal cells at the edge of the wound can



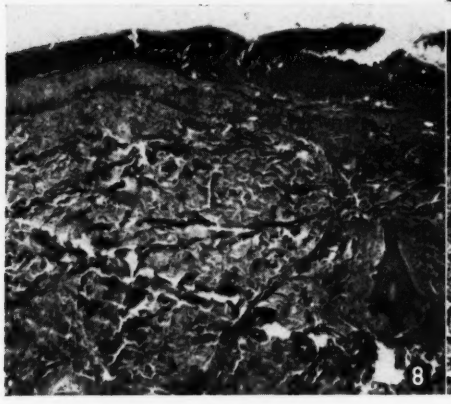


Fig. 7. A high power view of the epithelium newly regenerated from a neighbouring sweat gland in the middle of a donor site. The squamous character of the 2 or 3 layered regenerating epithelium is apparent, as is the extremely close contact of this epithelium with the denuded stratum reticularis of the dermis. Expecially noteworthy is the *absence of any new connective tissue below the new epithelium.* The 'crack', between the new squamous epithelium and the dense collagen fibres of the denuded dermis, is an artefact, but attributable to the poor attachment of the new epithelium to the underlying connective tissue. The original clot can be seen overlying the new epithelium. (PAH. × 440).

Fig. 8. Appearances of the edge of a 4-day-old donor site. The thickening of the untraumatized epithelium at the edge of the wound (at left) is now well marked, as is the newly regenerated epithelium covering the donor site (centre and right). The hair follicle (bottom right of this figure) was shown, by serial sections, to communicate with the surface at the point of greater thickening of the surface which is apparent near the right hand edge of this figure. Already, in this specimen, some new sub-epidermal exudate has become apparent towards the left hand edge of the donor site. (Ponceau-light green method. × 43).

Fig. 9. Five-day-old healing donor site. The marked thickening of the newly regenerated epithelium over-lying the donor site is apparent (at right). The epidermis at the edge of the donor site (left) is now even thicker than before and the junction between a regenerating sweat gland duct and the surface epithelium is also clearly depicted. Some keratinization of the thickening epidermis is now apparent. (EH and E. × 43).

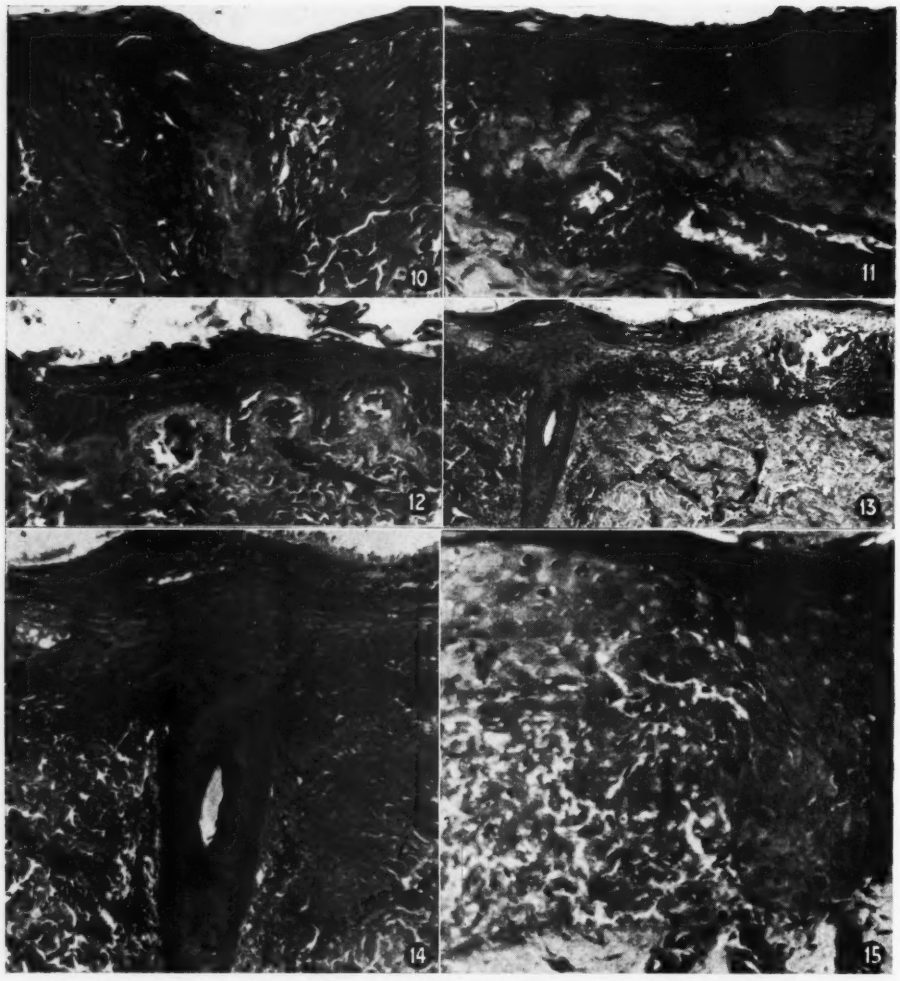


Fig. 10. The point of junction of the regenerating sweat gland and the new surface epithelium (seen low power Fig. 9) is here depicted. Notice the crowding of cells at the amputated orifice of the duct, the squamous nature of the new cells which pour out over the surface, and the immediate contact between these new surface cells and the underlying denuded stratum reticularis of the dermis at the fifth post-operative day. (PAH. × 200).
Fig. 11. Another view of the healing donor site at the fifth post-operative day. Once more the new squamous, surface epithelium is seen, and below it a band of glycogen-rich material stainable by the periodic acid-Schiff routine used for this specimen. Peri-vascular cuffing is well marked. (PAS. × 200).
Fig. 12. A view nearer the edge of the donor site than Fig. 11 to show the morphology of the new epithelium and its close contact with the underlying dermis. The epithelium seems to dip into the little crevices or valleys between the individual capillaries near the surface of the donor site. (H and E. × 200).
Fig. 13. Six-day-old donor site. Here, a hair follicle can be seen actively giving rise to new surface epithelium, which has now become considerably thicker than seen in the previous figures. This is the first time at which the sub-epithelial cell-free protein and glycogen-rich exudate has appeared, separating the new epidermis from the denuded dermis. (PAS. × 43).
Fig. 14. Higher power view of the regenerating hair follicle depicted in Fig. 13. Numerous mitotic figures were apparent in the upper layers of the epithelium lining the hair follicle at this time. The recently appearing sub-epithelial exudate, separating the new epithelium from the dermis, and the heavy peri-vascular cuffing, particularly in the vessels lying within the equivalent of the stratum papillaris, surrounding the hair follicle, are apparent. (H and E. × 100).

regularly be seen spreading below the covering blood clot, and in direct contact with the denuded stratum reticularis of the dermis. Also, in the sweat gland depicted in Figs. 4 and 35 it seems that epidermis, which is being regenerated from this duct, is now spreading along the surface of the dermis and *below* the overlying blood clot. Whereas in Figs. 4 and 35 it is clear that the epidermis has commenced to regenerate, even 24 hours after the initial operation, this regeneration of epidermis is not quite clear in Fig. 3, a picture taken at the edge of the wound. The spur of epidermis spreading from the edge of the wound, below the clot, may well be an artefact resulting from the removal or loosening of the epidermis at the time of the original operation. There is a very mild round-cell infiltration about the vessels deeper in the dermis of the wounded area. Perhaps the most striking feature of the dermis, at this time, is the alteration in the collagen fibres of the stratum reticularis immediately underlying the wound. This alteration in the fibres is shown in two ways:

i. The grouping of the fibres is changed as compared with the neighbouring dermal areas.

ii. The staining reactions of the fibres in the traumatized region have also altered, as indicated by the fact that they no longer stain intensely eosinophilic, but instead take up the basic dyes in the mixtures of stains used. In particular, these collagen fibres now stain diffusely and intensely purple with Mallory's phosphotungstic acid stain and not so intensely with the aniline blue of Mallory's triple stain. In fact, these altered collagen fibres stain with the orange G or the fuchsin of the Mallory's triple mixture. Perhaps the most striking feature of the staining reaction of the altered collagen fibres is the fact that they may become heavily stained following the application of methods usually employed for the depiction of elastic fibres. We have now shown that these tinctorial reactions are similar to those described in a recent Macy Foundation Conference (1950) as 'fibrinoid degeneration' of collagen and distinct from 'elastotic degeneration'.

Forty-eight hours after the initial operation the epidermis has already undergone profound changes, both at the margins of the excision and in the lining of the sebaceous and sweat glands and the hair follicles. Detailed changes in the epidermis at this time will be presented more fully below. However, even in low power views it is apparent that the epidermis at the edges of the wound has become very much thickened, as compared with its original state (*cf.* Figs. 5 and 6 with Figs. 1 and 2). It can be seen from Figs. 5, 6, 36-39 that not only has the epidermis thickened, but there has also been a profound alteration in its organization at the edges of the wound. Moreover, there seems to have been a definite shift of the *middle* layers of the epidermis across the denuded stratum compactum of the dermis. It should be stressed that this movement of epidermis takes place *below* the overlying clot and *in direct contact with the denuded stratum reticularis of the dermis in the wound area* (Fig. 7). In addition, it is apparent that there has been a marked increase in the round cells about the blood vessels even in the more superficial layers of the dermis (Fig. 6).

It cannot be too strongly stressed, at this point of the description, that the epidermis regenerating from the wound edges as well as from the mouths of the amputated glandular structure is in *direct contact with the denuded stratum reticularis of the dermis.* Contrary to expectations, and to the opinions previously expressed on this matter in the available literature, this newly regenerated epidermis is *not* separated from the denuded dermis by any exudate, either cellular or fluid, at *this* stage in the healing process. Apart from the round cell infiltration about the blood vessels and around the hair follicles and glandular appendages in the dermis, and the alterations in the staining reactions of the collagen fibres mentioned above, the dermal tissue in the wounded area remains strikingly inert, both at this stage as well as later in the healing process.

Figs. 8-12 show that the regeneration of the epidermis proceeds so actively during the *next 2 (4th and 5th) post-operative days,* that by the fifth day almost the entire denuded donor site has been covered by a thin layer of somewhat abnormal epidermal cells. The epidermal cells now covering the denuded area are derived largely from the middle layer (i.e.

Fig. 15. High power view of the edge of a healing donor site (sixth day) to show the alteration in the morphology of the original epidermis, the nature of the new epithelial cells flowing over the donor site, and the well marked separation of this new epithelium from the underlying donor site by a virtually cell-free exudate. It can also be seen, in this figure, that the adjacent dermis is devoid of any mitotic figures among the fibrocytes. The dark staining of the epithelial cells, at the surface and at the immediate point of junction between the original epidermis and the regenerating epidermis (at right), is due to the presence of large quantities of intracellular glycogen. (PAS. × 200).

stratum spinosum) of the original epidermis as well as from the rapidly regenerating epithelium which wells up over the raw surface from the skin appendages. The active participation of the epidermis, constituting the lining of these latter structures, is clearly evident from Figs. 10, 35, 36. In striking contrast to the regenerative activity of the epidermis, the dermis remains inert. Round cell infiltration about the dermal vessels is still very marked (Fig. 11); but apart from the accumulation of cells in the peri-vascular tissues, there is no evidence at all of regeneration on behalf of the fibrocytes normally associated with the compact fibres of the dermis. Consequently the close apposition of the regenerated epidermis with the denuded stratum reticularis of the donor site, and the absence of any intervening exudate, is again a striking feature of the histological appearance of specimens taken between the fourth and fifth post-operative days. This close apposition between the new surface epithelium and the stratum reticularis of the dermis is very clearly seen in Figs. 9 and 12. In Fig. 12 the newly regenerated epidermis can be seen to dip down between capillaries which lie near the surface of the denuded areas. In some specimens taken at this time a narrow band of sub-epidermal material may appear which stains fairly intensely with the periodic acid-Schiff method. A small number of round cells may be seen scattered in this glycogen-rich zone of sub-epidermal material (Fig. 11).

By the *sixth post-operative day* (Figs. 13-15) regeneration of new epithelium from the edges of the wound and from the numerous sweat glands and sebaceous glands is well advanced. (See especially Figs. 13 and 14). Even by the fifth day numerous mitotic figures were apparent in the sweat and sebaceous glands and in the hair follicles. However, by the sixth day mitotic figures have even appeared at the amputated mouths of the glands. As Figs. 13 and 14 show, the rather thick epidermis in the region of the sweat glands is now separated from the underlying dermis by quite a wide band of sub-epithelial exudate. This exudate (Figs. 14 and 15) does not proceed beyond the edges of the sources of regenerating epithelium, viz. the edges of the wound and the mouths of the ducts of the various skin appendages.

Thus, around the ducts of sweat and sebaceous glands, and from the edges of the wound whence epidermis is being regenerated, there lies a pool of proteinaceous, relatively cell-free fluid, almost in the form of a blister, separating the new epithelium from the originally denuded dermis of the donor site. The material constituting the sub-epithelial exudate is clearly rich in protein (as evident by the coagulation during fixation) and is also virtually devoid of cells (Fig. 15).

Fig. 15 reveals several important facts.

i. At the edge of the wound (from which this Figure has been taken) the epithelium has now thickened considerably and has a most unusual morphology.

ii. The completely inert character of the denuded dermis, even at this late time after the injury, is evinced by the absence of any mitotic activity in the fibrocytes of this tissue.

iii. The sub-epidermal or rather sub-epithelial fluid pool has obviously been coagulated by fixation, and contains only a small number of round cells within its substance.

iv. This fluid is rich in carbohydrates (possibly mucopolysaccharides), as shown by the strongly positive reaction with the periodic acid-Schiff routine. The glycogen-rich nature of the well-developed sub-epithelial exudate at the sixth day indicates that this is probably equivalent to the similarly located and similarly staining but less striking sub-epithelial layer described at the fifth day (Fig. 11). It is possible that this fluid may have been derived from lymphatics and has been trapped subepithelially when the denuded surface becomes completely covered by regenerated epithelium.

To *summarize* events from the time of operation to the sixth post-operative day (Fig. 47A, B), the most actively regenerating tissue in this denuded area is undoubtedly the epidermis, at the edge of the wound as well as that lining the skin appendages. Apart from round cell infiltration, which appears about the vessels from the first post-operative day, there is very little evidence of regenerative activity or any sign of activity at all in the dermal tissue underlying the wound. The regenerating epidermis thus spreads over the surface in close contact with the denuded dermis, and below the overlying blood clot. The blood shed at the time of operation apparently plays no part in the healing process, other than perhaps initially to 'protect' both the bared area and subsequently the thin layer of regenerating epithelium which spreads below this clot; nor does the dermis seem to participate at all actively in the healing process, at least not until the sixth post-operative day. The only other region which seems to participate at all in the healing process is the peri-follicular or

peri-glandular connective tissue which, as mentioned earlier, appears to be constituted of the same type of tissue as the sub-epidermal stratum papillaris of the dermis. The peri-follicular tissue, like the peri-vascular tissue, also develops quite a heavy round cell infiltration which may play some part subsequently, i.e. in events following on those occurring up to and including the sixth day.

By the *tenth post-operative day* the entire healing process seems to have changed. The regenerated epidermis has thickened very considerably, compared with the earlier stages in healing, while the sub-epidermal exudate has disappeared entirely and has been replaced by a completely new layer of young connective tissue. Not only has the epidermis (regenerated epithelium) thickened very markedly between the sixth and the tenth post-operative days, but it also seems to have developed very thick projections into the underlying new connective tissue. These thick projections, extending into the new connective tissue, have previously been mistaken by other authors as rete pegs. Comparison of Fig. 16 with Figs. 1 and 2 shows there is no morphological similarity between the 'true' original rete pegs, characteristic of the original skin of *this* area, and the projections of regenerated epidermis in the healing zone which are so striking at this time. Since (*vide infra*) these projections of regenerated epithelium into the new connective tissue ultimately disappear, we have preferred to call them 'pseudo-pegs' to distinguish them from the 'true' rete pegs of the original epidermis of the area studied. The development of such 'pseudo-pegs' in healing Thiersch graft donor sites and second degree burned areas has been clearly *depicted* by previous authors who have either not commented on them at all or have regarded them as regenerating 'true' rete pegs.[12, 15, 35] As indicated previously,[27] Brown and McDowell [12] have actually referred to these 'pseudo-pegs' of regenerated epidermis as regenerating *true* rete pegs. In fact, they went so far as to state that by the sixteenth post-operative day the skin has completely re-acquired its *normal* morphology. As will be shown, this is not in conformity with the facts gleaned from this study, and such remarks evince a lack of appreciation of the *original* micro-morphology of the skin field studied by various authors.

Apart from the marked hyperplasia of the newly regenerated epithelium, and the seeming invasion of the underlying regenerating, mesenchyme-like connective tissue by rather thick 'pseudo-pegs' of new epithelium, the other striking feature of the healing donor site at the tenth day is the appearance of new connective tissue *below* that layer of regenerated epidermis which had originally covered the wound by the sixth day, unattended by connective tissue regeneration. Close study of the dermis in this wounded area, even at the tenth day, reveals a complete absence of mitotic figures among fibrocytes lying in the originally denuded stratum reticularis of the dermis of the donor site. The only mitotic figures apparent in connective tissue in the healing site are those found in the immediately sub-epithelial zone. Since we have described and clearly portrayed the sub-epithelial accumulation of a proteinaceous, glycogen-rich and cell-free exudate, and since there is no evidence of mitotic activity in the fibrocytes of the stratum reticularis, we can only assume that the highly cellular new connective tissue, which appears by the tenth day below the thick regenerated epithelium, has been formed from the haematogenous round cells which have escaped from the circulation into that exudate which developed after the fifth day, and below the regenerated surface epithelium.

Very few new vessels are found in the sub-epidermal connective tissue at this stage and what vessels do appear have most primitive embryonic-like characters.

Thus healing activity is largely confined to the epidermis up to the sixth post-operative

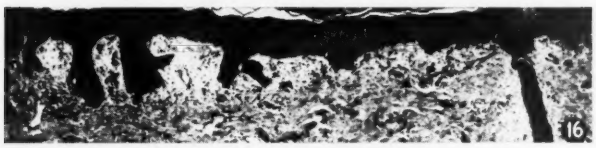


Fig. 16. Lower power view of a 10-day-old healing donor site. The extreme hyperplasia of the new epithelium covering the donor site (left half) is quite striking, as are the numerous thick 'invasive pseudo-pegs' of regenerating epithelium. The layer of originally cell-free sub-epithelial exudate is now filled with numerous round cells, some new blood vessels and actively migrating and mitosing fibroblasts. The point of contact between a regenerating sweat gland duct and the thickened surface epithelium is also shown. (H and E. × 100).

day; and between the sixth and tenth days there appear, rather suddenly, in a new sub-epidermal exudate, round cells and thereafter new connective tissue cells of a fibroblastic nature together with some simple blood vessels.

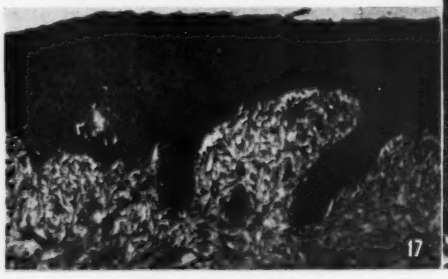
After the tenth post-operative day the most obvious changes in the healing process are those in the sub-epidermal area. This is evident by the marked thickening of this layer which now separates the denuded stratum reticularis of the donor site from the new surface epithelium. The most striking changes in this new sub-epithelial connective tissue are the rapid accumulation of numerous fibroblastic cells and lymphocytes together with the infiltration, into this new connective tissue, of delicate thin-walled new capillary vessels. It is clear from Figs. 17 and 18 that these new capillaries seem to be developed from the perithelium of blood vessels originally lying close to the surface of the stratum reticularis of the donor site (Fig. 12). Moschcowitz[65] has provided strong evidence that lymphocytes also participate in angiogenesis. Thus the only components of the dermal connective tissue which participate actively in the healing process, up to this stage, are the blood vessels and white blood cells. Activity, in relation to blood vessels, was evidenced at the outset by the accumulation of numerous round cells about these blood vessels, the loosening of the perithelial tissue, which becomes metachromatic to toluidin blue, and subsequently the budding of new capillaries into the unusual exudate which has appeared sub-epithelially between the fourth and sixth days.

With the increasing cellularity of this new sub-epidermal zone of regenerating connective tissue, the epithelium itself undergoes further hyperplasia (Figs. 17, 18). The epidermal 'spurs' or 'pseudo-pegs' themselves become simpler, blunter and more sharply defined from the surrounding connective tissues. As indicated in Figs. 27-30, the sub-epidermal region now becomes rich in reticulin fibres, but at this time only a limited number of very delicate collagen fibres can be detected by special stains. With the accumulation of reticulin fibres in this sub-epidermal connective tissue, the cellularity apparent by the tenth and even the seventeenth days becomes progressively less, while the thin-walled capillaries, apparent even until the tenth day, become consolidated and thick walled.

Three distinct layers are now apparent in the healing zone (Fig. 47C):

1. The thick hyperplastic regenerated epidermis with numerous thick 'pseudo-pegs'.
2. The rather cellular new sub-epidermal connective tissue rich in reticulin fibres and fibroblasts.
3. The virtually inert stratum reticularis of the denuded dermis below (2).

Between the 17th and 48th post-operative days changes supervene which can only be regarded as a type of re-organization of both the new connective tissue and of the regenerated epithelium (Figs. 47D, E). *Inter*



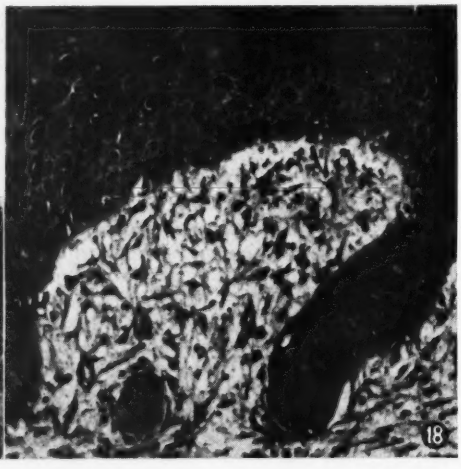


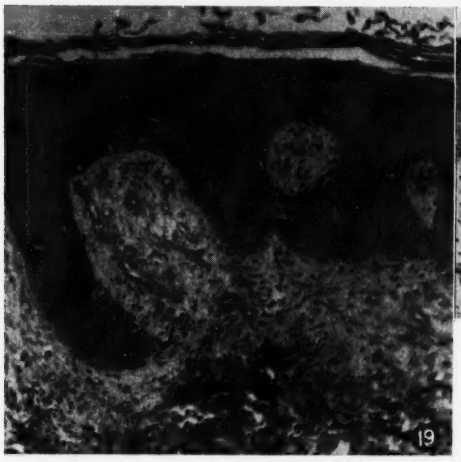
Fig. 17. Moderately high power view of the surface of a healing donor site at 17 days. The very marked epithelial thickening, the change in the morphology of the cells from squames to almost cuboidal or polyhedral prickle cells with irregularly distributed intracellular glycogen, the diminution in the glycogen content of the new epithelium, as compared with that at the sixth and tenth post-operative days, the thinning and rounding of the tips of the pseudo-pegs invading the new connective tissue, and the great cellularity and mesenchymal-like appearance of this new sub-epidermal tissue are all clearly depicted. (PAS. × 100).

Fig. 18. High power view of centre portion of the donor site depicted in Fig. 17 to show the nature of the new sub-epidermal connective tissue, the flattening of the epithelial cells at the point of contact with the new connective tissue, and the morphology of the regenerated epidermis. (PAS. × 100).

alia, the epidermis becomes thinner, the 'pseudo' rete pegs become surrounded by numerous lymphocytes and plasma cells, and collagen fibres now appear within the new connective tissue in the sub-epidermal zone. The extensive spurs of new epithelium seen projecting into the regenerated connective tissue, as late as the seventeenth to the twentieth day, now become eliminated by separation from their overlying tissue and are subsequently surrounded by numerous round cells, foreign body giant cells as well as fibrocytes. Thus within the fibrosing sub-epidermal connective tissue numerous separated islands of epithelium may be found undergoing internal keratinization and inducing foreign-body reactions. It is difficult to differentiate these islands of epidermis from the typical epithelial 'pearls' so clearly described in association with squamous cell carcinoma of the skin.

That the 'pseudo-pegs' of epidermis, extending into the new connective tissue, are in fact nipped off or separated from the overlying epithelium, is substantiated by 2 facts. (i) In serial sections these islands can now be seen to be completely independent of the overlying epidermis and of any skin appendage; (ii) The line of junction between the new epidermis and the regenerated sub-epidermal connective tissue is gradually straightened out. These phenomena are seen in Figs. 19-21, 47D, 47E.

By the 48th post-operative day all the excessive invaginations of epidermis into the underlying connective tissue have been eliminated and consequently the new epidermis takes up the appearances of 'scar-like' epithelium. Moreover the sub-epithelial connective tissue has now developed a considerable quantity of collagen, while the reticulin fibres have simultaneously diminished in numbers. Small epithelial pearls, surrounded by typical foreign-body giant cell reactions, may be found scattered in the new sub-epithelial connective tissue for many months, if not years after the original trauma. The epithelium in the Thiersch graft donor site has not acquired its normal morphology even by the eighth *year* after the taking of the graft; nor has the dermis acquired its normal morphology. (*cf.* Figs. 23 and 24).



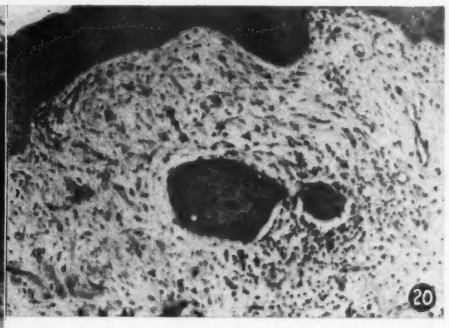


Fig. 19. Morphology of the new epithelium covering the donor site, in another experimental subject, showing once more the profound alteration in the morphology of the epithelium at the seventeenth post-operative day, compared with earlier specimens. The surface layers of the new epithelium have become squamous once more, the basal cells are approaching a columnar form, the rather irregular thick pseudo-pegs depicted in Fig. 16, at the tenth post-operative day, are now becoming thinner and rounded in form while the new connective tissue has already become less cellular and more fibrous. Primitive blood vessels in the new sub-epidermal tissue are apparent. Below this layer of new connective tissue, degenerated and morphologically altered elastic fibres of the original dermis are apparent. (Stained Verhoef, H and E. × 100).

Fig. 20. Morphology of the healed donor site in a third subject at the seventeenth post-operative day. The epithelium has already altered, in this subject, to approach a scar-like epidermis; the surface layers are squamous, the epithelial-connective tissue junction has altered its morphology and become much straighter than in previous specimens, while the round cell infiltration related to the originally invasive pseudo-pegs is also clearly depicted. These pseudo-pegs become separated off from the parent surface epithelium, undergo internal keratinization, as can be seen commencing in this figure, and subsequently evoke severe foreign body reactions with ultimate fibrosis. (EH and E. × 100).

CONNECTIVE TISSUE CHANGES IN HEALING THIERSCH GRAFT DONOR SITES

Since Wolbach's classic paper (1933) disclosing the effects of vitamin C deficiency on the regeneration of fibres in connective tissue, the relations between matrix and fibre formation have received considerable attention from cytologists and histochemists. We have paid particular attention to the study of fibrillogenesis in order to disclose more precisely the relationship between the regeneration of epithelium and of connective tissues during the healing process.

It is apparent that until the fifth to sixth post-operative days there is very little, if any, deposition of fibres, even of reticulin fibres. Fig. 25 depicts the normal relationship between collagen, reticulin network and overlying epidermis in the non-traumatized skin of the antero-medial aspect of the arm. In suitable preparations, immediately below the epidermis, there lies a fine feltwork of *reticulin fibres* with argyrophil loops which

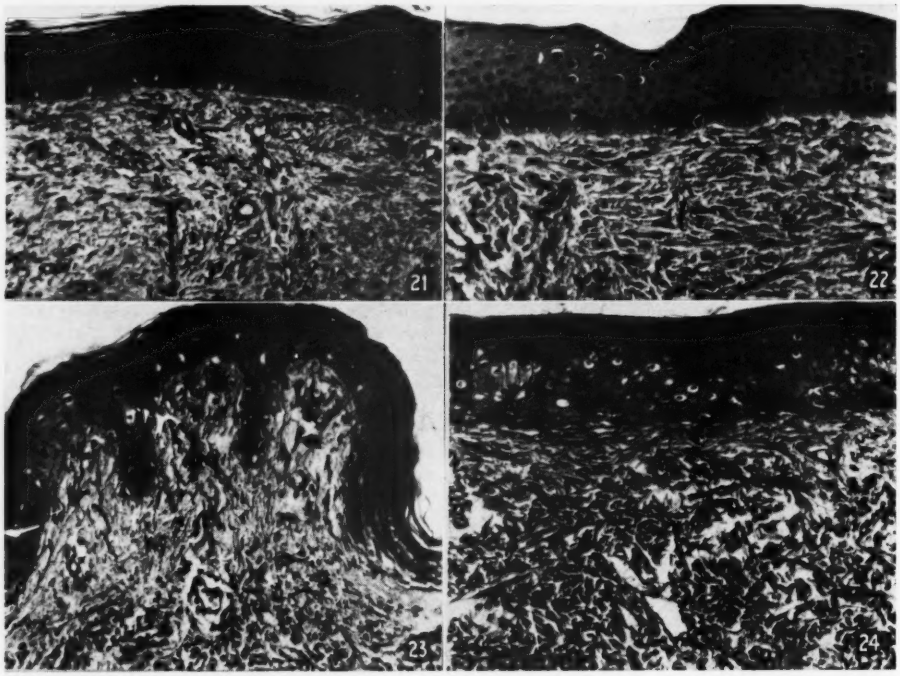


Fig. 21. Morphology of the epithelium over a healed donor site 21 days post-operatively. The rather squamous, thick, highly keratinized epidermis is apparent, but particularly noteworthy is the almost straight line of junction between the new epithelium and the now rather fibrous, less cellular new connective tissue. The epithelium, at this time, closely resembles scar epithelium. Also striking, is the fact that the epithelium in this specimen is considerably thinner than previously described (*cf.* Figs. 16 and 17), and that the morphology of the epithelial cells and their general arrangement has also altered profoundly. (PAS. × 100).

Fig. 22. Forty-eight-day-old healed donor site. Here the straight epidermal-dermal junction is striking as are also the profound alterations in the epithelial morphology, the reappearance of a thin stratum granulosum in the surface layer of the epidermis, and the very highly collagenized new connective tissue which has developed in the donor site. The absence of the stratum papillaris and of fine elastic fibres in this new connective tissue is clearly depicted. (Stained Orcein H and E. × 200).

Fig. 23. Morphology of the normal skin abutting an 8-year-old healed donor site. The morphology of the epithelium, the poorly developed rete pegs, the delicate sub-epidermal elastic tissue network, and the thickness of the stratum papillaris are all clearly shown. (Stained Orcein, H and E. × 200).

Fig. 24. Eight-year-old healed Thiersch graft donor site. The scar-like nature of the epidermis now covering the healed donor site, the abnormality of the epidermal-dermal junction, the absence of a true stratum papillaris and of any rete pegs, and the paucity of fine elastic fibres in the immediately sub-epidermal zone are all clearly shown. Orcein H and E. × 200).

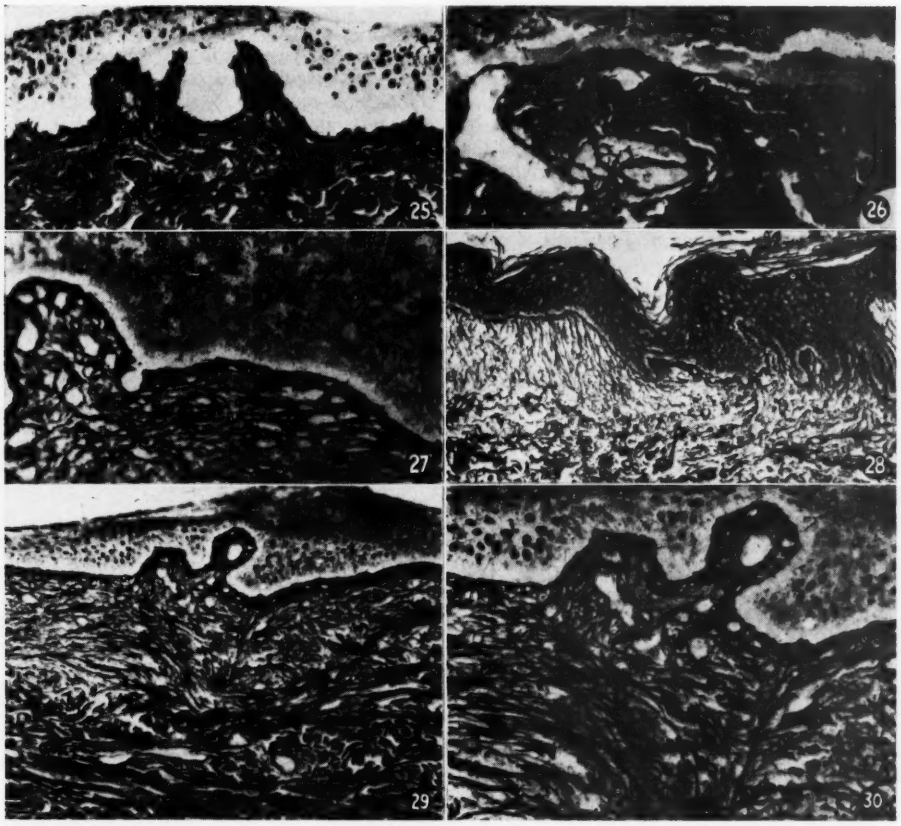


Fig. 25. Untraumatized skin abutting on donor site 24 hours after operation. Reticulin fibres in the stratum papillaris, and their irregular projections into the overlying epidermis, are clearly demonstrated. These fine hair-like processes, extending into the overlying epidermis, are apparently identical with the reticulin loops described by Odland (1950). A fine sub-epithelial reticulin meshwork in the stratum papillaris clearly distinguishes this layer from the deeper lying stratum reticularis of the dermis. (Wilder reticulin method. × 200).

Fig. 26. Donor site 24 hours post-operatively. Picture taken from same section as that in Fig. 25. It can here be seen that the stratum papillaris and part of the stratum reticularis of the dermis have been removed, there is no evidence of reticulin regeneration as yet, and, at left of figure, a narrow pale grey zone can be seen separating the dermis from the overlying clot. This narrow zone is the newly regenerated epithelium. (Wilder reticulin preparation. × 200).

Fig. 27. Showing the well marked sub-epithelial reticular meshwork which is developed below the new epidermis by the tenth post-operative day. (Wilder reticulin method. × 200).

Fig. 28. Reticulin preparation of 17-day-old healing donor site showing line of junction between the untraumatized epithelium at right and the healed donor site at left. The absence of rete pegs in the healed area and the different organization of the reticulin fibres distinguishes the healed donor site from the untraumatized skin in which well marked rete pegs and a distinctive sub-epithelial fine reticulin meshwork are present. (Wilder preparation. × 100).

Fig. 29. The increase in the reticulin fibres in the zone between the newly regenerated epidermis and the denuded stratum reticularis of the dermis at the eighth day, is here well depicted. Apart from a few projections of the new connective tissue into the overlying epidermis, there is no evidence of rete peg formation such as was clearly present at the tenth post-operative day. Note also that the orientation of fibres in this figure is quite strikingly parallel to the overlying epidermis. (Wilder preparation. × 100).

Fig. 30. High power view of the middle of Fig. 29 to show, in detail, the epithelial-reticulin junction. It can be seen that the hair-like projections of reticulin (intra-epithelial reticular loops of Odland) have not yet reappeared. (Wilder preparation. × 200).

project into the overlying epidermis in the manner described by Odland.[69] Fig. 26, on the other hand, portrays the appearances of the denuded donor site at 1 day, where the pale line of regenerated epithelium can be seen in direct apposition with the coarse bundles of collagen fibres of the stratum reticularis of the dermis, and without any intermediary feltwork of reticulin fibres. Particularly noteworthy is the absence of any reticulin loops projecting into the thin, newly regenerated layer of overlying epidermis, covered by the thick granular layer of coagulated blood.

The reticulum preparations thus confirm the previous observations on similar sections of skin stained by other methods. The absence of the sub-epidermal reticulin network originally present and of any signs of new connective tissue formation below the already regenerated epidermis is quite striking up to the sixth day. By the *tenth* day, however (Fig. 27) there is a distinctly definable thick layer of new reticulin fibres underlying the epidermis and separating it from the deeper stratum reticularis of the dermis. The general arrangement of this reticulin network, its thickness and the absence of any projections of reticulin fibres into the new, overlying epithelium is again striking. By the *seventeenth day* (Fig. 28) the differences in the pattern of the sub-epidermal reticulin feltwork become even more apparent on comparing this layer in the *normal* untraumatized epidermis, with that below the newly regenerated hyperplastic epithelium covering the wounded area. In the non-traumatized skin (right of Fig. 28) the sub-epidermal reticular layer is relatively thin, while the fibres seem to follow the line of the undulating rete pegs. There is a distinct basement-like membrane of reticulin fibres following the basal layer of the untraumatized epidermis. The reticulin network underlying the *regenerated* epithelium (which now covers the wounded area) is much thicker than normal, and the fibres seem to course in a totally different direction, viz. *perpendicular* to both the denuded stratum reticularis and to the overlying regenerated epithelium. There is, however a distinct, continuous line of sub-epidermal reticulin which appears to form a basement-like membrane comparable with that seen in the untraumatized skin at the right of Fig. 28.

After the seventeenth day and, in some specimens, even before this time, the reticulin fibres tend to run parallel to the overlying healing epidermis rather than perpendicular to it. There is a distinct, although narrow, compactly arranged zone of reticulin fibres immediately below the new epithelium, although this layer is in no way comparable with that originally depicted in Fig. 25. A comparison of Figs. 25 and 30 reveals (i) a greater width of the new sub-epithelial reticular layer in the healed site as compared with that of the normal untraumatized skin; (ii) the greater thickness of the individual reticulin fibres in the healed area; (iii) loop-like projections of reticulin fibres into the overlying newly regenerated epithelium are still absent.

Collagen fibres were extremely rare until about the eighth to tenth day. By the seventeenth day these aniline-blue and van Gieson positive fibres have become much more abundant in the broad new connective tissue zone which now separates the regenerated epithelium from the originally denuded and apparently still inert stratum reticularis of the dermis. With the progressive increase in the number of collagen fibres, and the associated diminution in the reticulin fibres originally deposited, by the seventeenth day the entire new sub-epithelial connective tissue layer changes its pattern as well as its relationship both to the regenerated epithelium and to the stratum reticularis of the dermis. Another striking feature of this connective tissue layer, at this time, is the virtual absence of those 'pseudo-pegs' which were so apparent in the earlier biopsy specimens (Figs. 16-20). The 'amputation' of the pseudo-pegs and their apparent ultimate removal by the active foreign-body reaction which they evoke (Fig. 20), lead to a straightening of the line of contact between the newly regenerated epithelium and the regenerated connective tissue.

With the removal of these abnormal projections ('pseudo-pegs') of epithelium, with the increase in collagen fibres and with the progressive thickening of the individual fibres, the entire organization of the regenerated sub-epithelial connective tissue layer changes. Even as late as the forty-eighth day (Fig. 31) although the reticulin basement-like membrane in contact with the new epithelium is rather thicker than at earlier stages in the healing process, nevertheless there are only a few rather stubby projections of reticulin fibres into the overlying epithelium.

This absence of intra-epithelial projections of reticulin is a feature of healed split-skin graft donor sites for a much longer time than has hitherto been recognized, as is substantiated by Figs. 32-34. At 2 years after removal of the graft (Fig. 32) the epidermis is still somewhat thicker than that originally

present in the donor site and its morphology is still abnormal (see below). The immediately sub-epidermal basement-like membrane of reticulin fibres is still thicker than that observed in the original skin (*cf.* Fig. 32 with Fig. 25), and it is devoid of the many small reticulin loops which were apparent in Fig. 25. Fig. 33 represents the appearances of normal skin of the abdomen from which a Thiersch graft had been cut 8 years before biopsy. In the normal epidermis in this figure the thin sub-epidermal basement-like membrane of reticulin fibres is once more apparent, as are the numerous fine projections of these reticulin fibres into the overlying epidermis. On the other hand, the 8-year-old abdominal Thiersch-graft donor site, abutting on the normal skin depicted in Fig 33, has a totally different arrangement of epidermis, reticulin basement-like membrane and sub-epidermal reticulin fibres. The fine felt-work of sub-epidermal reticulin fibres of the stratum papillaris of the normal dermis, apparent in Fig. 33, is absent from the sub-epidermal region in Fig. 34. In fact the stratum papillaris has failed to regenerate in the donor site even 8 years after the removal of the graft. So too, have the fine intra-epidermal projections of reti-

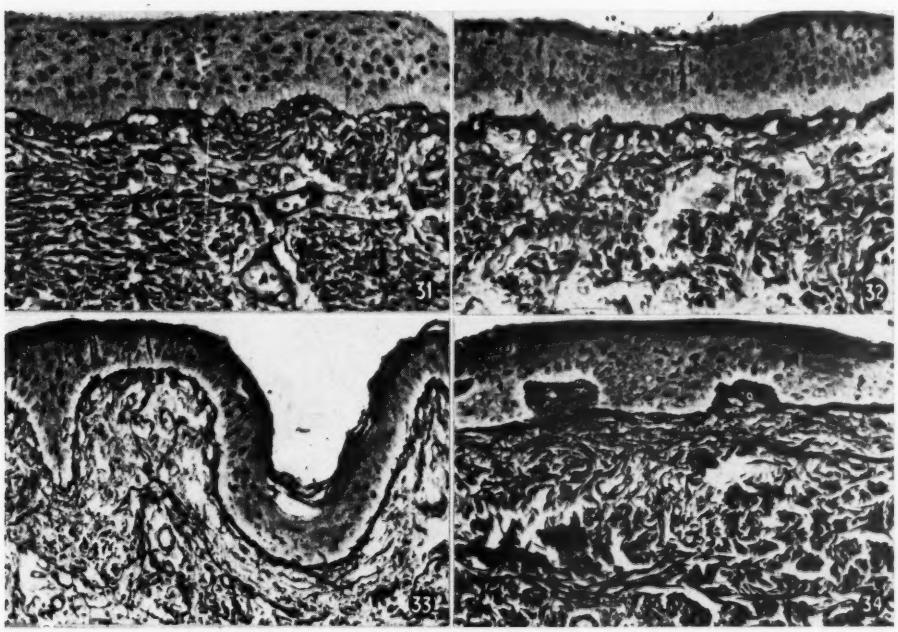


Fig. 31. Forty-eight-day-old graft donor site. At this time the entire pattern of organization of the sub-epithelial reticulin network has changed considerably as compared with that seen at the seventeenth and eighteenth post-operative days. There is the slightest suggestion of the reappearance of the reticular loops projecting into the overlying new epidermis. However, these loops are, at this stage, extremely short and fine. The line of junction between the epidermis and the new connective tissue has now straightened considerably, and there is virtually no sign of the regeneration of rete pegs. (Wilder preparation. × 200).

Fig. 32. Appearances of a 2-year-old healed Thiersch donor site. The immediately sub-epithelial reticular network has condensed considerably, as compared with earlier preparations, and the intra-epithelial reticular loops are now slightly more prominent than previously. Once again attention should be drawn to the fact that there is no evidence of the regeneration of the stratum papillaris or of rete pegs. (× 200).

Fig. 33. Appearances of the reticulin of untraumatized skin neighbouring a Thiersch graft donor site. The characteristics of the epidermis, its rete pegs, and its junction with the underlying stratum papillaris are graphically demonstrated. The nature of the sub-epidermal reticular network is also clearly depicted. Fine intra-epidermal reticular loops are clearly evident at intervals along the length of this stretch of epidermis. (× 200).

Fig. 34. Appearances of reticulin, and of epidermal-dermal junction in an 8-year-old healed graft donor site abutting the normal skin shown in Fig. 33. The scar-like nature of the epidermis is once more apparent, only a few rather blunt connective tissue projections into the overlying epidermis can be seen, while the reappearance of extremely fine and short intra-epidermal reticular projections are also apparent. (Wilder preparation. × 200).

culum failed to regenerate, while the basement-like membrane of reticulin fibres immediately underlying the epidermis is much thicker and more regular than that seen in the untraumatized skin depicted in Fig. 33. Careful scrutiny of a biopsy of this 8-year-old healed abdominal donor site reveals the presence of very fine, stubby reticulin projections into the scar-like epidermis now covering the healed donor site. Since these loops of reticulin, projecting into the epidermis in the normal skin, constitute an important part of the anchorage of the epidermis to the underlying dermis,[69] it is not surprising that, as is general clinical experience, the epidermis covering 'true' scars is easily detachable, even by slight trauma. Whereas in an *incisional* scar, these reticulin loops projecting into the epidermis are usually totally absent, the healed Thiersch graft donor site does acquire *some* equivalent of the original projections, albeit these are poorly developed. This may, in part, account for the fact that the epidermis overlying a healed Thiersch graft donor site is far more firmly adherent to the underlying connective tissue than is the case for other types of scars.

The main points disclosed by the foregoing analysis of the mode and nature of the connective tissue regeneration during the healing of a split-skin graft donor site, may be *summarized* (Figs. 47C-47E) as follows:

1. Critical studies of the development of reticulin fibres reveal that these do not develop in the wounded area for 4-6 days after injury.

2. Reticulin fibres first appear only *after* the regenerating epithelium has covered the denuded dermis.

3. Reticulin and collagen fibres, and the associated connective tissue cells, only develop *after* the regenerating epithelium from the skin appendages has, for the main, established continuity along the denuded surface.

4. This regenerating connective tissue does not develop in the shed blood, but in a new exudate which develops sub-epithelially *after* the denuded stratum reticularis of the dermis has been covered with the first layer of new epithelium.

5. The connective tissues, once they have commenced to regenerate, exert a marked

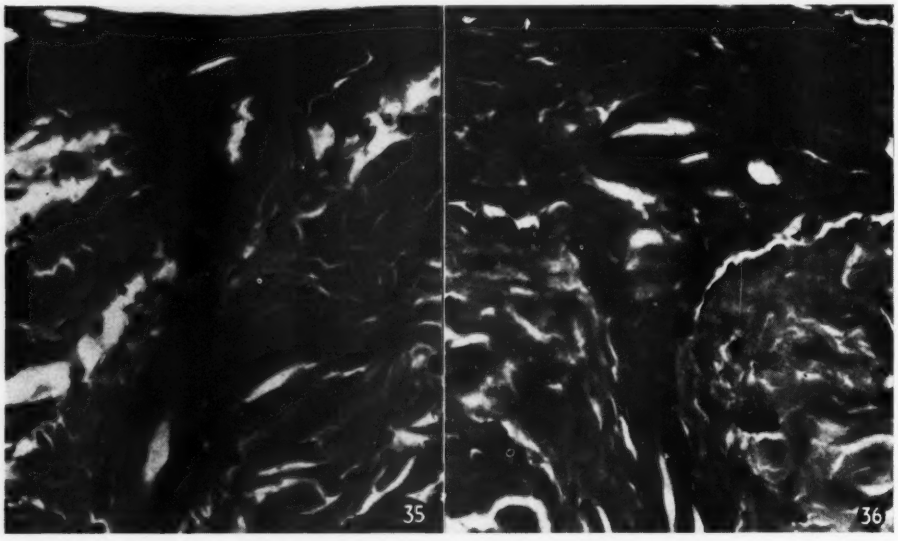


Fig. 35. High power view of the regenerating sweat duct shown in the centre of a donor site in Fig. 4. The absence of any evidence of connective tissue regeneration at the surface of the donor site is here strikingly apparent. The active regeneration of epithelium at the new mouth of the amputated sweat gland can be seen to result in some crowding of the new cells, which are spreading in an umbrella-like fashion beneath the overlying clot. Considerable mitotic activity was detected in serial sections of this same sweat duct. (H and E. × 440).

Fig. 36. High power view of a regenerating sweat duct in a 2-day-old healing Thiersch graft donor site. Again it can be seen that there is no sign at all of connective tissue regeneration at the junction between the overlying clot and the denuded dermis. The umbrella-like spreading of new epithelium, being generated from the sweat duct beneath the clot and in direct contact with the denuded dermis, is strikingly demonstrated. (H and E. × 440).

regulatory effect on the subsequent development of the new epithelial covering of the wound.

6. The fibre pattern of the untraumatized dermis is not re-established even 8 years after removal of a split-skin graft. This is well shown in the abdominal graft illustrated in Figs. 23 and 24.

CHANGES IN THE EPIDERMIS DURING THE HEALING OF THIERSCH GRAFT DONOR SITES

According to many investigators the anchorage of epidermis to dermis depends upon the connective tissue-epithelial relationship as expressed, on the one hand by the organization of the reticulin (and perhaps even of the elastic) fibres[63] and, on the other hand, by the morphology and arrangement of the basal layer of epidermal cells.[69] We have therefore also paid some attention to the mode of regeneration as well as to the re-organization of the epidermis at various times during the healing of Thiersch graft donor sites.

We have seen, so far, that:

1. Within 24 hours of injury, regeneration of epidermis has commenced.

2. Epithelium is regenerated from the following sites:

(a) From the wound edge (Figs. 3-6, 8, 9, 15 and 38).

(b) From the ducts of sebaceous and sweat glands and from hair follicles. Epithelium from these sources pours out from the amputated ends of ducts and spreads across the dermis in all directions around their orifices (Figs. 10, 13, 14, 35 and 36).

(c) As new epithelial cells, generated from these several sources, pour over the bared area, in direct contact with the denuded stratum reticularis of the dermis, the various streams, flowing from many directions, run together to form a continuous, albeit thin, epithelial covering to the wound. Only after this stage, does a distinct sub-epithelial exudate develop which now separates the new epithelial cells from the compact dermis upon which these cells originally lie.

Following the appearance of this blood-free, sub-epidermal exudate, lymphocytes and monocytes emigrate from the circulation into this proteinaceous, glycogen-rich sub-epidermal pool. The regeneration of connective tissue now commences and progresses rapidly. With the appearance of this stratum of connective tissue, the overlying epidermis becomes extremely thick, and broad, irregular pseudo-pegs extend into the new connective tissue layer which, as yet, is relatively free of collagen fibres.

Subsequently these pseudo-pegs of epithelium become surrounded by a heavy infiltration of round cells and fibrocytes and, in due course, these spheres of epithelial cells are separated from their overlying parent surface layer. These epithelial spheres then undergo internal keratinization, forming epithelial pearls. The epithelial pearls, in turn, ultimately evoke foreign-body giant cell and fibrocytic reactions from the new connective tissue, and are thus slowly eliminated over a period of months, if not of years. 'Amputation' of the epithelial pseudo-pegs results in a levelling of the basal portion of the regenerating epithelium. After several months or years the dermo-epidermal junction becomes virtually straight, resembling scar epithelium.

We have already shown that the connective tissue, in the healed donor site, does *not* regain its original pattern of organization. The same holds true for the epidermis, which is scar-*like* in its morphology 2 and even 8 years after the removal of the graft.

Origin and Development of the Epithelium Covering Thiersch Graft Donor Sites. Since the regeneration of epithelium commences soon after injury, it is essential to obtain frequent biopsies *during the first few days after injury* for a precise understanding of the origin of the first epithelial cells which cover the denuded area. The first epithelium which spreads from the wound edge across the denuded stratum reticularis of the dermis is derived from the upper layers of the stratum Malpighi of the original epidermis (Figs. 6, 7, 11, 12, 15, 38, 40 and 42). This holds true also for the new epithelium generated from sweat ducts and especially from hair follicles. Despite the complexities of studying the epidermal layers when these are arranged in tubular form, it seemed quite apparent that, in these appendages too, new epithelium was generated from the equivalent of the stratum spinosum. During the earliest days of epithelial regeneration, both at the wound edges and in the skin appendages and glands, the majority of mitotic figures were consistently in the middle rather than in the basal layers of the epidermis. Only after the new epithelial cells had already acquired contact with the denuded dermis, were mitotic figures found in the *basal* layers of the new epithelium (Figs. 38, 40). At the sixth day numerous mitotic figures were seen in the stratum spinosum of the hair follicle depicted in Figs. 13 and 14.

Initially, the cells comprising the upper layers of the stratum Malpighi (and immediately below the stratum granulosum) seemed to cascade dermal-wards and then to spread wedge-like *beneath* the overlying blood clot (Figs. 6, 9, 39). These almost flat cells of the upper layers of the stratum spinosum of the original untraumatized epidermis retained their disc-like form and their intercellular bridges as they appeared to migrate, or to be pushed across, the epithelial gap occasioned by the taking of the graft. This explains the squamous characters of, and the presence of prickle cells in the new epithelium which overlies the wound during the early stages of epithelial regeneration (Fig. 40). Only 8-10 days

after the injury, when the sub-epithelial connective tissue is regenerating, does the new epithelium thicken markedly and change its morphology.

The epithelium originating from glands and hair follicles regenerates in a manner very similar to that growing from the edges of the wound. In fact, the entire circumference of each of the many residual ducts and hair follicles may justifiably be regarded as minute but multiple equivalents of the edges of the donor site. This is clearly evident in Figs. 4, 10, 16, 35 and 36 where, once more, squamous cells can be seen pouring from the ducts and appendages across the denuded stratum reticularis of the dermis. As in the case for the epithelium growing from the wound edges, so too the epithelium generated from the ducts of glands and from hair follicles is first separated from the denuded dermis by the appearance, sub-epithelially, of a proteinaceous and glycogen-rich exudate between the fourth and sixth days. Epithelium arising from the ducts also becomes thick and loses its squamous

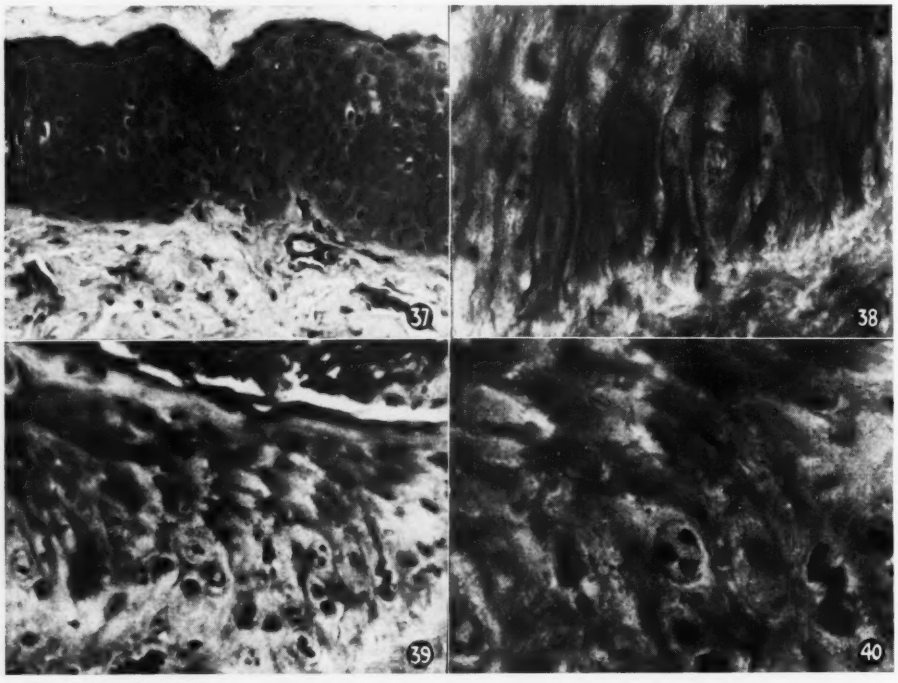


Fig. 37. Moderately high power view of epithelium in untraumatized skin neighbouring a Thiersch graft donor site. The epithelium is approximately eight to ten layers thick, some of the cells are low cuboidal, there is a distinct zone of 2 or 3 layers of stratum granulosum, no stratum lucidum, and a fine feathery stratum corneum. These are the appearances of a normal epidermis of the antero-medial aspect of the arm in the subjects studied during these investigations. (H and E. × 200).

Fig. 38. High power view of the epidermis abutting the edge of a donor site 48 hours after operation. The epithelium has now adopted a tall columnar form, the protoplasmic process extending into the underlying stratum papillaris are clearly shown, while tonofibrils can be seen extending almost from the basal cells well into the upper layers of epithelium in which prickle cells are numerous. (PAH. × 1,000).

Fig. 39. High power view of the epidermis at the edge of the Thiersch graft donor site at 2 days post-operatively. The newly regenerating epidermis can be seen extending across the donor site which lies towards the right of this figure. Numerous mitotic figures can be seen (lower right of figure) and it is also apparent that the cells spreading across the donor site are being derived mainly from the upper layers of the stratum spinosum. (PAH. × 440).

Fig. 40. Very high power view of the lower right hand portion of the field depicted in Fig. 39 to show the large numbers of mitotic figures which are present in the uninjured epidermis *immediately* abutting the donor site, and extending across the healing area. The alterations in the morphology of the epidermis, in this area next to the donor site, are also apparent in comparison with Fig. 37. (H and E. × 1,000).

characters only *after* the new connective tissue has developed.

It should be mentioned here that, in conformity with the observations of Bishop, it seemed that the loose peri-glandular and peri-follicular connective tissue became as cellular as the perithelial tissue around blood vessels. It seemed to us that the reactions of this loose connective tissue are comparable with those seen in the stratum papillaris of the dermis at the wound edges and around the blood vessels. It is from *this* loose connective tissue, relatively rich in mucopolysaccharides, that round cells seem to emigrate into the sub-epithelial exudate. Subsequently fine capillaries also develop from this same source.

Morphological and Histo-Chemical Changes in the Epithelium Covering the Denuded Donor Site. Initially only a thin layer of epithelial squames spreads across the donor site (Figs. 3-12, 35, 36). At this stage, while the epithelium is still closely applied to the denuded stratum reticularis of the donor site, it (the epithelium) *cannot* be regarded as being *attached* to the underlying connective tissue. This is particularly well shown in Fig. 7 where the several layers of flat epidermal cells can be seen separated, even by a fixation artefact, from the denuded stratum reticularis of the dermis. Occasionally during the taking of the biopsies a small amount of blood was found to have seeped in from the edges of the biopsy specimen, and to have separated this thin, new, squamous epithelium from the underlying dermis. After the third day the new epithelium thickens, but still retains its squamous form and simulates very closely the morphology of the upper few layers of the stratum spinosum of the normal epidermis (Fig. 37); so much so, that these squames seem to have been derived *entirely* by the migration of the upper layers of the epidermis of the wound edges. This is not so because numerous mitotic figures can be seen among these squamous cells near the wound edge (Figs. 39, 40). That some of these squamous cells round off and mitose is evident even at the second day (Figs. 39, 40).

By the third day the epithelial cells which have migrated from the wound edge still retain their squamous form, and 'prickles' can usually be distinguished clearly around the basal cells lying in contact with the stratum reticularis of the donor site (Fig. 42).

Even as late as the fifth or sixth day, when

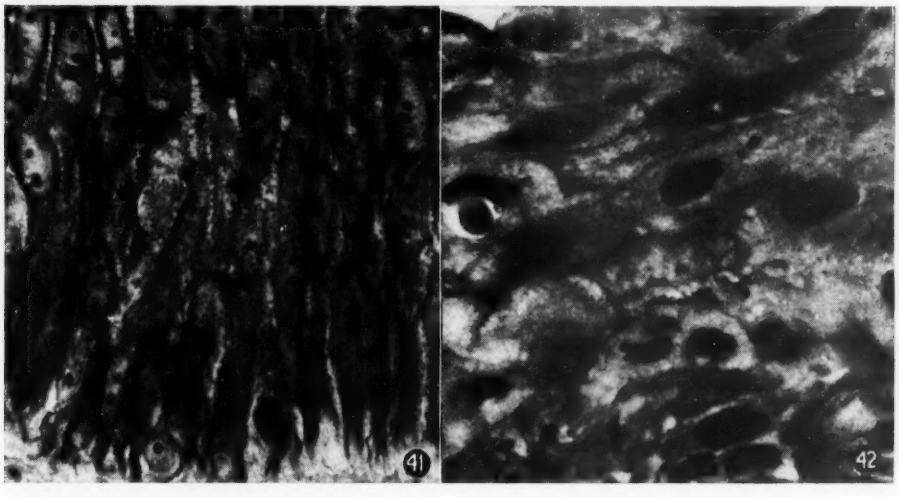


Fig. 41. Very high power view of untraumatized epithelium abutting the Thiersch graft donor site, at the third post-operative day, to show, once more, the neighbouring epidermis, the distinct and numerous fine, epithelial protoplasmic processes extending into the upper portion of the stratum papillaris of the dermis, and the greater number of prickle cells in the epidermis. Prickle cells can now be detected even in the most basally situated epithelial layer. (PAH. × 1,000).

Fig. 42. Newly regenerated epithelium overlying the 3-day-old healing Thiersch graft donor site. The new epithelial cells are squamous in form, closely packed, with fine but distinct inter-epithelial cell bridges. The absence of any evidence of sub-epithelial connective tissue regeneration is also once more apparent. (Verhoef elastic method. H and E. × 1,000).

the newly generated epithelium is only 4-6 layers thick, most of the cells still retain their original squamous form (Figs. 11-14).

With the development of the connective tissue in the exudate which forms below the new epithelium, the morphology of the latter changes rapidly and profoundly. The originally flat cells round off; numerous 'prickles' or intercellular bridges appear in *all* the layers of the new epithelium; large amounts of intracellular glycogen are now apparent in almost all the layers of regenerated epithelium overlying the wound as well as in the cells near the edges of the wound and lining the hair follicles. Accumulation of glycogen is most marked in the 2 or 3 uppermost layers of the new epithelium. A stratum granulosum is still absent.

With this increase in the thickness of the epithelium and with the alteration in its morphology and histochemistry, thick pseudo-pegs seem to invade the rapidly developing sub-epithelial embryonic-like connective tissue which now separates the epithelium from the stratum reticularis of the dermis. These changes progress during the period from the seventh to the eighteenth days. Particularly noteworthy, at this time, when the new epithelium has thickened considerably, and cells have lost their original squamous form, is the development of large numbers of prickle-cells. In fact, between the fifteenth and forty-eighth days almost every cell throughout the thickness of the new epidermis has acquired prickles.

By the twenty-first day some of the basal cells have become distinctly columnar and this alteration in the form of the basal cells of the new epithelium is even more apparent by the forty-eighth day (*cf.* Figs. 43 and 44). Only in specimens obtained from healed donor sites about 2 years after the graft was taken, can distinct differentiation between basal and spinosum layers be considered to have occurred.

Thus the epithelium which covers the graft donor site commences as a few layers of flat squames, then thickens to acquire many layers of broader squames which in turn alter into numerous layers of small polyhedral prickle cells. Shortly after the development of this thick epithelium, with broad pseudo-rete pegs invading the underlying new connective tissue, and simultaneously with the development of collagen fibres in this sub-epithelial connective tissue, the *basal* cells of this thick new epithelium acquire a columnar form. Differentiation of a distinct columnar basal layer of epithelial cells can only be said to have occurred by the forty-eighth day, in the specimens studied by us. Even at this stage, however, the basal cells, while columnar in form, retain numerous prickles (Fig. 44). The first specimen available to us, in which the number of prickles among the basal cells seemed to have diminshed, was one removed from a 2-year-old, healed donor site. It was only in this specimen that clear differentiation could be made (on the basis of the arrangement and of the varying morphology of epithelial cells) between the basal layers, the 'true' stratum spinosum and the squamous portion of the stratum spinosum as it merged into the overlying stratum granulosum and stratum reticularis.

Comparison of Regenerated Epithelium with Original Untraumatized Epidermis. The morphology of the original epidermis is shown in Figs. 1, 2 and 37. The epidermis is constituted of 8-12 layers of polyhedral, rather closely clustered cells (Fig. 37). The stratum granulosum consists of only 1-2 layers, the stratum lucidum is absent, whereas the stratum corneum is thin and delicate. In the untraumatized epidermis of this skin field, the basal layers are very low columnar and do not show any well developed cytoplasmic processes extending into the underlying dermis. However, the epidermis adjoining the excised area reveals, by the second day, that the basal layer of cells has rapidly adopted a tall columnar or pyriform appearance with extremely well developed cytoplasmic processes extending into the upper layers of the stratum papillaris of the dermis. Not only have these basal layers become columnar in shape but (Fig. 38) the lower 3-4 layers of the stratum Malpighi are columnar. The tonofibrils are well developed (as can be shown quite clearly with Mallory's phosphotungstic acid haematoxylin stain) and extend through 3 or more layers of cells. The basal cells are especially interesting, particularly because of their well developed cytoplasmic processes. These protoplasmic processes of the basal epithelial layers appear to interdigitate with the loops of reticulin fibres,[69] thus providing the attachment of the epidermis to the underlying stratum papillaris of the dermis. There is no well-developed or distinct basement membrane.

These changes, which supervene in the untraumatized epidermis adjoining the Thiersch donor site, for a distance of 2-3 mm. away from the wound itself, are maintained for a number of days after the initial injury. Fig. 41 is a further example of the appearances of the untraumatized epidermis near the wound edge. The tall columnar appearance of the

lower 3-4 layers of the epidermis is well demonstrated, as are also the well-marked protoplasmic processes extending into the underlying dermis from the basal layers of cells. In this specimen (biopsy on third day) the tonofibrils are even better developed than on the second day. Consequently prickle cells are a prominent feature of the untraumatized epidermis and these cells extend almost from the basal layer to the surface of the epidermis. This prominence of prickle cells in the skin of the area under study is a most unusual phenomenon and can be related directly to the healing processes.

These alterations in the morphology of the epidermis, involving a change from polyhedral

Fig. 43. Epithelium overlying a 21-day-old healed donor site. It can be seen, that in comparison with earlier specimens, the newly regenerated epithelium overlying the donor site has now altered considerably in morphology. The basal cells have attained a relatively tall columnar form and there are numerous prickle cells commencing almost at the basal layer. The uppermost layers of cells in this newly regenerated epidermis have changed their morphology and have acquired a polyhedral form together with numerous inter-cellular bridges. There is no evidence of protoplasmic processes extending from the basal epithelial layer into the new sub-epidermal connective tissue. The basal epithelial cells seem to be joined together by a distinctly staining basement-like membrane. (Iron haematoxylin. × 1,000).

Fig. 44. Appearances of epithelium in a 48-day-old healed Thiersch graft donor site, to show the profound alteration which has supervened in the new epithelium. The basal cells are now low columnar, there is a slight suggestion of new cytoplasmic processes extending from this basal epithelial layer into the underlying new connective tissue, and prickle cells are now well marked, extending from the basal layer to the surface. (Iron haematoxylin. × 1,000).

Fig. 45. Appearances of epithelium from a 2-year-old healed graft donor site. The basal epithelial processes have now reappeared (*cf.* Figs. 43 and 44), although these processes are now much more numerous, finer and shorter. The basal epithelial cells are now low columnar or pyriform. Prickle cells are extremely numerous, even in the basal layer, and extend to the uppermost layers of the epithelium. (Iron haematoxylin. × 1,000).

to well-marked columnar epithelial cells, are consistently observed during the healing of all the cutaneous wounds studied by us. This same alteration in the morphology of epidermal cells can also be seen regularly during the early stages of carcinogenesis induced by the application of methylcholanthrene. These findings suggest that one of the early responses of epidermal cells close to sites of injury is a morphological change from a polyhedral or cuboidal to a columnar stratified epithelium. The appearance of intercellular tonofibrils, with the consequent prominence of prickle cells even in basal layers is also apparently a prominent change associated with the repair of epidermis shortly after an injury, whether due to physical or chemical irritants. These morphological changes in the basal layers of untraumatized epidermis may persist for 20-48 days after the initial injury.

Changes in the epithelium, which pours over the donor site from the wound edges and from the ducts of glands during the first few post-operative days, have already been mentioned (Figs. 38, 42). However, we were specially interested in determining whether the regenerated epidermis ever recovered its original morphology and, if so, how long after injury. By the twenty-first day the new epithelium, now overlying the donor site, has approached, in form, that found at the edges of the wound. A few columnar cells have appeared at the base and prickle cells are numerous (Fig. 43). However, at this stage the *basal* cells do *not* have protoplasmic processes extending into the underlying dermis. Instead the bases of the neighbouring cells seem to be joined together by a rather tenuous line of material which stains in the same way as the cytoplasm of the basal cells themselves. By the forty-eighth day the epithelium has thinned somewhat, the basal cells are not so tall, the protoplasmic 'bridges' between their bases are still present, but there is some indication of the beginnings of protoplasmic processes. However, these protoplasmic processes from the basal cells do not yet extend into the underlying connective tissue (*cf.* Fig. 44 with Figs. 38 and 41).

Two years after the taking of the Thiersch graft, the epithelium overlying the donor site (Fig. 45) is quite different from both the original epidermis (Fig. 37) and from that just described in Figs. 43 and 44. By the second year the basal cells have become very low columnar and have developed protoplasmic processes which now extend into the underlying connective tissue. These processes are much finer, more numerous and much shorter than those described before.

Even 2 years after the initial injury the regenerated epidermis is still abnormally rich in prickle cells and the organization of the uppermost layers differs strikingly from that of the original epidermis (*cf.* Figs. 45 and 37). These abnormalities in the epidermis in the injured area may persist for as long as 8 years after the graft was taken.

To summarize these changes in the epidermis, it can be said: The untraumatized epidermis is characterized by 8-12 layers of relatively small polyhedral cells and comparatively devoid of prickle cells. The basal layer is relatively devoid of protoplasmic processes such as those described by Odland. However, within 24-48 hours of the injury the entire morphology of the epidermis at the edge of the wound changes, with the appearance of tall columnar cells extending upwards for 4-6 layers above the basal cells. The basal cells themselves are tall columnar and have well marked protoplasmic processes extending into the underlying stratum papillaris of the dermis. Prickle cells, and the associated tonofibrils, which were not a prominent feature of the original epidermis, now become apparent and persist in the untraumatized epidermis adjoining the wound for a period of 24-48 days.

The regenerated epidermis which covers the denuded area is initially constituted of squames, apparently derived from the upper layers of the uninjured neighbouring epithelium. This is borne out by the presence of intercellular bridges even from the first appearance of this new epithelium over the denuded surface. With the passage of time the original flat squamous cells become more numerous and later become polyhedral with numerous intercellular bridges. The basal layers of the newly regenerated, originally squamous epithelium soon become columnar, but protoplasmic processes extending into the underlying dermis are absent for at least 48 days after the injury. Prickle cells extend almost to the basal layers. Only after 2 years, when the epithelium has reconstituted itself somewhat, do the cells revert almost to their original polyhedral form, and the basal layers of epithelium acquire protoplasmic processes such as those described by Odland as binding the epidermis to the underlying dermis. Even at 2 years prickle cells are far more prominent and extend further down in the epidermis than was characteristic of the untraumatized epidermis.

DISCUSSION

Many interesting topics arise from the foregoing analysis of the finer anatomy of the healing of wounds involving loss of only part of the total thickness of the skin. We have chosen only the following for brief consideration, in the light of the available literature:

1. The origin and development of the regenerated epithelium.

2. The origin and development of the components of the regenerated connective tissue.

3. Epithelial-connective tissue relations during wound healing.

4. Possible implications of these findings for understanding the pathogenesis of skin cancers.

5. Some practical applications of these findings.

1. THE ORIGIN AND DEVELOPMENT OF THE REGENERATED EPITHELIUM

Eichenlaub and Osbourn [19] have recently reviewed critically much of the available literature concerning epidermal regeneration, in the light of their own studies on the histogenesis of the epidermis in human embryos. They concluded that:

'Our findings in this preliminary study . . . indicate . . . that the prickle cells are more actively concerned with proliferation than the basal cells. There is some evidence that the prickle cells may be the primary functional cells of the epidermis proper . . . It appears probable that we should not consider that the prickle cells are derived only from the basal layer, nor that only the latter layer is normally capable of mitosis. Rather we should consider the lowermost layers of prickle cells as constituting a true stratum germinativum. Thus we consider prickle cells more concerned with proliferation and the basal cells less concerned with it.'

This view is in accord with Hartwell's original findings (1928) and with the opinions expressed by Mills in discussing Hartwell's publication. Montgomery [65] also agrees with these views. Our findings add further support to the opinions expressed by Eichenlaub and Osbourn, since both our morphological and histological findings reveal that the first epithelial cells to cover the donor site were derived from the upper 3-4 layers of the stratum germinativum of the epidermis adjoining the wound, as well as from the equivalent of these layers in hair follicles and sebaceous gland ducts.

The accumulation of glycogen in these upper layers, and the equivalent thereof in the hair follicles, is one of the earliest irrefutable signs of regenerative activity in the epidermis at the edge of the wound. This histochemical change is soon followed by the migration of the glycogen-laden squames across the wound, deep to the overlying clot and in direct contact with the exposed stratum reticularis of the dermis. Increase in glycogen, change in shape and increasing mitotic activity are apparent in this order, further and further from the wound edge, as the epidermis surrounding the defect becomes progressively involved in the healing process.

The morphological changes in the epidermis overlying the healing donor site are almost identical in this repair process with those described and portrayed by Scothorne *et al.*[84, 85] in the epidermis of autografts. However, the first specimen described by these authors was obtained only *five* days after grafting. We have been able to study the changes supervening *during each of the first 5* post-operative days, and can state that glycogen accumulation in individual cells is most marked during days 2 to 4, diminishing progressively thereafter. Whereas Scothorne and Scothorne describe a normally appearing epidermis in autografts by the fifteenth day, the epithelium in the healing donor site does not approach normality even 2 years after the injury. This finding is in striking contrast with that described in healing Thiersch graft donor sites by Brown and McDowell,[12] who state (p. 65):

'Healing is complete by the sixth day, and by the ninth day, conversion to squamous epithelium is so complete that the papillae are formed and some keratin is being thrown off.'

In the legend to their Fig. 39, p. 64, *op. cit.*, they state:

'Ninth day, with normal appearing epithelial surface. Rete pegs beginning to develop and keratin being thrown off.'

Our own findings have fully confirmed our original descriptions and opinions [27] that this view, expressed by Brown and McDowell, and

supported by others (e.g. Converse and Robb-Smith [15]), is not in conformity with more detailed and more prolonged studies of the healing of split-skin graft donor sites. The marked thickening of the epidermis which supervenes after the sixth day, at a time when the new sub-epithelial connective tissue is first seen, results in the appearance of *pseudo*-pegs invading this new connective tissue. We can confirm the opinion of both Brown and McDowell and of Converse and Robb-Smith [15] that the epidermis thickens considerably between the eighth and fifteenth days, but we *cannot* agree with any of these authors that this epithelium is 'normal' in appearance or approaches the morphology of 'normal epidermis' by the ninth or tenth day. The thickening of the epidermis, at this time, is associated with the invasion of the underlying connective tissue by thick pseudo-pegs, and we have shown that the ultimate disappearance of these epithelial 'invasions' of the dermis ('pseudo-pegs') is closely bound up with the development and maturation of new sub-epithelial connective tissues. Even when relatively thin split-skin grafts have been removed, the regenerated epithelium does *not* regain the original morphology of the epidermis removed with the graft. On the contrary, the new epidermis in the healed donor site, 2 years and longer after the removal of the graft, is quite abnormal and closely *simulates* that usually regarded as characteristic of scars. However, the regenerated epidermis covering the healed donor site is ultimately *not* identical with that found in old scars. The former becomes thinner, develops basal protoplasmic processes and a stratum granulosum, and may be described as lying midway in morphology between the original epidermis and scar-like epithelium.

These microscopic findings are in conformity with careful clinical study of healed Thiersch graft donor sites, which reveal definite and persistent differences between the epidermis covering healed donor sites and that of the neighbouring untraumatized skin, described previously.[27]

Also interesting is the manner in which the migrating squames of the upper layers of the abutting stratum germinativum penetrate between the denuded stratum reticularis of the dermis and the dry firmly adherent blood clot. Clark and Clark,[14] in another of their outstanding, almost dramatic studies of the regeneration of tissue in observation chambers prepared in living animals, provide ample evidence to demonstrate that regenerating epithelium is apparently capable of secreting proteolytic enzymes which may dissolve fibrin. These authors draw attention (pp. 193-194) to the fact that:

'... the fibrin quickly disappeared in the region next the *differentiating* epidermis and for a considerable distance lateral to it. The dissolving of the fibrin occurred within 24 hours after the appearance of an easily recognised epidermal strand—and it progressed as differentiation advanced. In cases in which differentiation of layered epidermis occurred before the arrival of new blood capillaries, dissolving of fibrin took place only on the side away from the basal layer . . .

Unlike the effect of the newly circulating blood capillaries upon the surrounding medium, the dissolution of the fibrin next the granular layer of epidermis was not followed by the formation of semi-gelatinous tissue substance. Instead, the area of fluid increased until, with the formation of a complete epidermal wall, it became a cavity, or lake . . .'

Our observations on fixed specimens clearly confirm the precise descriptions provided by Clark and Clark from observations *in vivo*. We have demonstrated, in sections, that the regenerating epithelium appears capable of insinuating itself like a wedge between the blood clot and the denuded dermis. Since, as can be shown by clinical observations, the blood clot which forms on the surface of a graft donor site is firmly adherent to the underlying dermis for some days, the invasion of the epidermis between the clot and the dermis must involve some ability, on the part of the epidermal cells, to liquify the protein fibres binding the clot to the dermis. This hypothesis is in conformity with the observations of Clark and Clark. The pool or 'lake' of fluid described by these authors in their observation chambers may, in fact, be identical with that observed by us as appearing *below* the newly generated epithelium between the fourth and sixth days. Quite apart from allowing the epidermis to penetrate between the clot and the dermis, this 'liberation' or accumulation of fluid between the newly regenerated epithelium and the dermis seems to play an important part in the subsequent regeneration of the connective tissue. It may well be that in the healing process the epidermis plays an important role, not only in covering the dermal defect but, by its proteolytic action on the clot and perhaps on some of the upper fibres of the dermis, in providing a new, proteinaceous and glycogen-rich fluid medium into which the new connective cells may grow and thrive.

These are only some of the very fascinating problems arising from the study of epidermal regeneration in healing wounds. The possible

implications of these findings for an understanding of the factors regulating regeneration of both epithelium and connective tissues in the healing of all wounds in tissues, as well as in the genesis of carcinoma, more particularly in the skin, cervix and stomach, will now receive attention.

2. THE ORIGIN AND DEVELOPMENT OF THE COMPONENTS OF THE REGENERATED CONNECTIVE TISSUE

Most textbooks dealing with the various phases of the repair process state quite categorically that new connective tissue is regenerated by the mobilization of fibrocytes previously present in the dermis neighbouring the injured tissue. Hartwell[38-41] was quite definite in the view that 'in wounds in human beings it is evident that the macrophage is the true fibroblast'. This confirmed views originally propounded by Macklin,[57] Maximow,[61] and subsequently confirmed by many other investigators, including Moschcowitz.[66] Our own previous studies on the healing of incised wounds, in both animals and in man,[27-30] also support this view. Howes and Harvey,[44] analysing the results of many previous studies on the healing of wounds in experimental animals, state:

'... fibroplasia starts abruptly at the fourth day ... This (their evidence) suggests that one is dealing with a basic phenomenon common not only to all tissues but to all animals tested and leads one to question the commonly accepted view that the fibroblast involved in healing of a wound is derived by differentiation from the already differentiated fibroblasts of the fixed tissues involved ... We want to make the point clear, if we can, that the concept we have is *not* that the primary healing of the wound is due to cells derived from adjacent fixed tissues, but from cells which appear in the early exudative process, and which, by growth or differentiation, become fibrous cells, and that the scar tissue itself is not a differentiated connective tissue, but is the result of a process specific in itself.'

Howes and Harvey subsequently maintained that 'the first phase, that of fibroplasia, originates in and from resting, relatively undifferentiated cells closely related to the "mesoblast"'.

In a previous extensive review of the nature of one of the most controversial problems in histology, haematology and pathology—the origins and morphology of the most primitive connective tissue cells in adult organisms—we drew attention to the fact that such 'mesoblasts' or 'reticulum cells' cannot easily be distinguished in fixed or even in imprint preparations of adult connective tissues. In these previous investigations we stressed particularly the pluri-potentiality of many adult connective tissue cells, under suitable local environmental conditions.[23] Our present investigations on wound healing indicate beyond doubt that neither in incised nor in excised wounds do the fibrocytes, originally present in the traumatized dermis, become active or mobilize and undifferentiate into fibroblasts. All the available evidence from the study of incised and excised wounds indicates that the new fibroblasts, which develop during the healing of connective tissues in cutaneous wounds, are derived primarily from cells which have migrated from the circulation, or which have been derived from the peripheral tissue surrounding blood vessels and the *residual* portions of the stratum papillaris of the dermis about hair follicles, etc. This view is further substantiated by the present study.

In addition to the problem of the origin of the first fibroblasts in the healing of cutaneous wounds, we studied the generation of the fibres in the new connective tissue. Like many others, we failed to obtain satisfactory morphological evidence of the origin of the reticulin and collagen fibres during the early phases of wound healing. However, we have been able to provide some documentation about the time of origin and subsequent development of reticulin and of collagen fibres in the new connective tissue in the healing donor site (*vide supra*).

There is some controversy about the time of appearance of the first elastic fibres in healing donor sites. Converse and Robb-Smith[15] observed the first elastic fibres as fine fibres 5 weeks after the initial injury. In the legend to their Fig. 17, a photomicrograph of a 32-day-old healed donor site, they state:

'The elastic fibres of the original dermis can be clearly seen and very fine elastic fibres have developed in the new formed sub-epidermic connective tissue.'

Bishop[10] stated that elastic fibres had not appeared even 7 *weeks* after initial injury. Our own findings did not reveal new elastic fibres in the regenerated connective tissue even after 2 *years*. However, careful examination both of the traumatized dermis underlying the healing tissue, as well as of the new connective tissue which develops between the denuded stratum reticularis of the dermis and the regenerated epidermis, revealed several interesting phenomena. It was common to find, even as early as after 48 days, coarse clumps of irregularly arranged elastic fibres related to the hair follicles and to the blood vessels damaged at the time of graft removal. By the

forty-eighth day it was apparent that the traumatized *collagen* fibres, originally present in the stratum reticularis of the dermis, had undergone marked changes, with the appearance, microscopically, of plaques of coarse fibres, staining with all the traditional elastic stains here used, and which had apparently been derived from the original collagen by traumatically induced chemical changes. This phenomenon has previously been described by us both in relation to the healing of grafts,[27] as well as in association with the development of various types of skin carcinomata in man.[28-30] Such an alteration in the collagen fibres, manifested by thickening of the individual fibres, increased affinity for elastic stains, as well as by other distinctive tinctorial reactions, has been named by us 'elastotic degeneration' of collagen. In a previous publication[30, 31] we have attempted to provide some criteria for identifying 'fibrinoid' degeneration as well as elastotic degeneration of collagen on the basis of its peculiar tinctorial reactions. It would seem that these fibres bear a close relationship to those originally described by Unna[92] as 'collacin' and 'elacin'. We propose to detail our findings regarding such 'elastotically degenerated' collagen fibres in a future report.

We did not detect new elastic fibres in regenerated connective tissue even after 2 years. There were a few fine elastic fibres in the new sub-epidermal connective tissue 8 years after removal of the graft. However, the fine sub-epidermal network of elastic fibres, characteristic of the normal stratum papillaris of the skin, had not appeared even by the eighth post-operative year.

3. EPITHELIAL-CONNECTIVE TISSUE RELATIONSHIPS DURING WOUND HEALING

The conventional view of healing excised wounds is shown diagrammatically in Fig. 46. Most authorities state quite categorically:

'In order to migrate, epithelium requires a substratum of mesenchyme. If the mesenchyme remains intact, epithelium may begin migrating at once... If, however, the mesenchyme has been disrupted, as in deep wounds of the skin, epithelium is unable to migrate until a new mesenchymal substrate is built up by granulation tissue. Thus occurs a delay of several days, after which epithelium spreads on the granulation tissue, passing between this and the scab. It is noteworthy that epithelium spreads not on top of but beneath the scab, and therefore in contact with the *newly formed mesenchyme*.' (Anderson, 1953, p. 50. Italics inserted).

Forbus[21] states, in dealing with the healing of 'destructive wounds':

'If the defect involves other tissue than the epithelium, it must be filled before the surface can be covered. This is accomplished through the proliferation of fibrous tissue and blood vessels in the wounded tissue. The wound usually is first filled, or at least is covered, by an exudate of fluid, fibrin, and wandering cells. This serves as a scaffold for the subsequent growth of fibroblasts and blood vessels, which is closely correlated. The growing connective tissue and vessels extend into the superficial layer of fibrin and little by little create a new and fully organised tissue . . . This combination of growing blood vessels and fibroblasts, fibrin, and macrophages and other wandering cells is called granulation tissue . . . When the granulation tissue fills the defect, or nearly so, the proliferating epithelium at the margins of the wound extends over the surface and gradually covers it.' (p. 894).

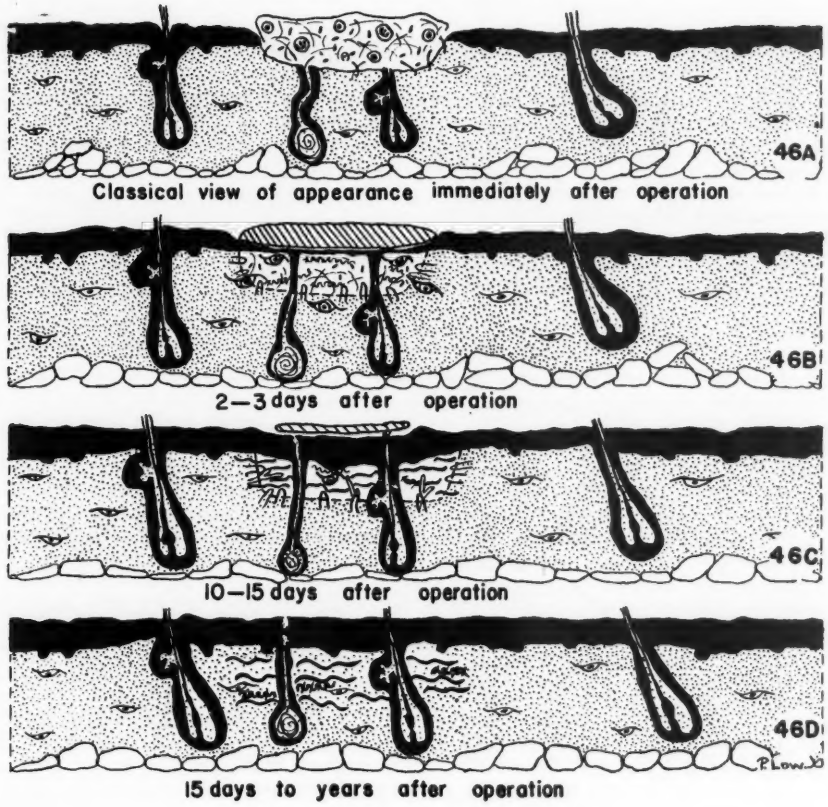
In an outstanding study of the healing of self-inflicted wounds, Bishop[10] arrived at similar conclusions. He states, in several places, the following:

'. . . a spread of epithelium from the hair follicle waits upon the growth of a mound of granulation around it . . . one factor determining the rate of epithelization again appears to be the development of a fresh granulation base . . . The degree of maturation of granulation tissue appears to determine the time of covering by epithelium . . . It seems obvious that the overall latency of epithelization is the time required by tissue injured to various depths to regenerate a suitable surface for epithelium to grow over, and implant upon, rather than being determined by an essential delay in initiating marginal growth . . . Epithelium heals the wound surface only *after* granulation tissue has matured to a stage permitting implantation of migrating epithelial cells.'

We have quoted these authorities to make quite clear the generally accepted views about the relative roles of epithelium and of connective tissue during the early phases of the healing of excised wounds. These same opinions have also been generally expressed by authorities quoted on the subject of the healing of *incised* wounds.[28-30]

Since our own findings (derived from the study of extensive human and animal material) are in total contradiction to the above-quoted views, we have been meticulous in our attempts to clarify this aspect of healing processes. We have therefore made special efforts to acquire specimens from healing wounds *daily during the first few post-operative days*. A most careful analysis of this material has revealed the foregoing findings, summarized in Fig. 47.

In broad outline we may state that the *first* tissue to show any signs of regeneration and to *grow* actively across a defect in the skin is *not* the connective tissue (as stated by the authorities quoted), but the epithelium. We have described in detail the earliest epithelial growth and have attempted to define precisely the plane along which this epithelium grows. In conformity with generally expressed



Key to symbols used in Figs. 46 and 47.

=New Blood Clot.	=Scab.
=Emigrated Leucocytes.	=Reticulin Fibres.
=New Capillaries.	=New Collagen Fibres.
=Original Fibrocytes.	=New Elastic Fibres.
=Mobilized Fibroblasts.	=Epithelial Pearls.
=Original Dermal Collagen.	=Giant Cells.

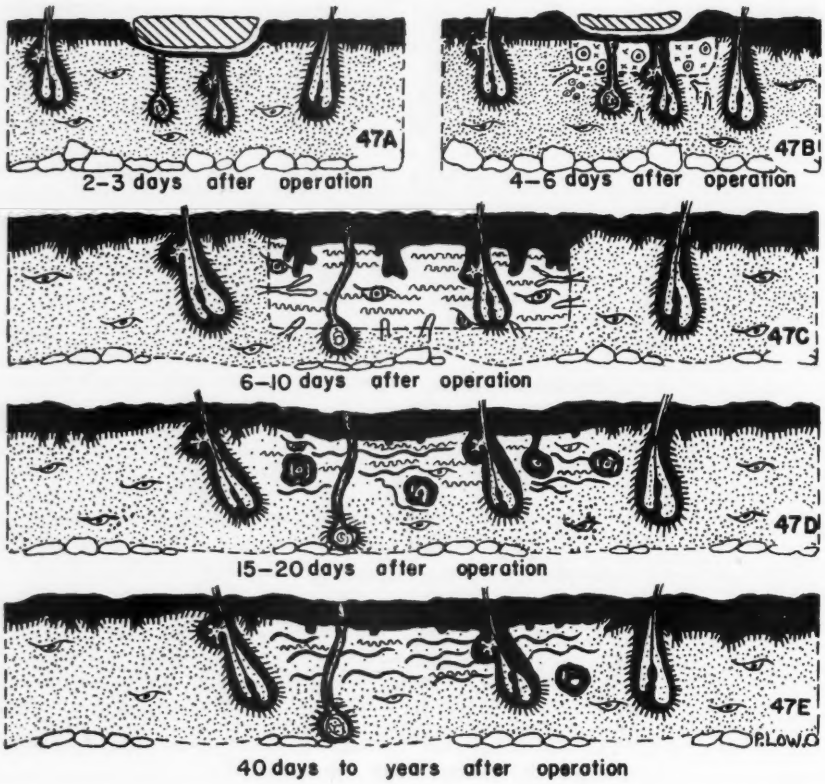
Diagrams illustrating *generally held views* on the healing of wounds involving partial thickness skin loss, as represented by the uncomplicated healing of thin split skin graft (Thiersch graft) donor sites (see text for full discussion).

Fig. 46*A*. Appearances immediately after operation, showing, depth of excision, blood clot in donor site with residual portions of glands below.

Fig. 46*B*. Two to 3 days post-operatively showing overlying 'scab', connective tissue alleged to be regenerating in clot or in serum exuded therefrom, regrowth of epithelium from wound edges and from residual glands, etc. in the donor site. How the epithelium from the latter sources reaches the surface *through* the pre-formed surface 'granulations' is *not* clearly explained. Connective tissue is alleged to regenerate by mobilisation of fibrocytes in neighbouring dermis.

Fig. 46*C*. Appearances 10 to 15 days post-operatively with 'normally appearing' epidermis with rete pegs and early fibrosis of 'granulations'.

Fig. 46*D*. After the fifteenth post-operative day, the donor site is reported as acquiring the morphology of completely normal skin with perhaps some slight evidence of dermal fibrosis. Elastic fibres are reported as reappearing by the third week.



Diagrammatic summary of the authors' findings on the healing of Thiersch graft donor sites (partial skin loss excised wounds).

Fig. 47*A*. Within 2 to 3 days of operation the epithelium regenerating from the wound edges and glands remaining in the donor site, has covered the denuded stratum reticularis of the dermis. Note that the stratum papillaris of the dermis (subepidermal oblique hatching) has been removed with the graft (*cf.* Figs. 1 and 2—photomicrographs). There are no signs of connective tissue regeneration at this time, apart from peri-vascular round cell cuffing in the adjacent dermis.

Fig. 47*B*. Four to 6 days post-operatively, the denuded surface has been completely epithelialized and a new sub-epithelial exudate is starting to appear. Into the latter, round cells are emigrating from the peri-vascular cuffing and from the blood stream. There is *still virtually no connective tissue regeneration.* Thus, *the epithelium from amputated glands and hair follicles reaches and covers the raw surface before there is any connective tissue regrowth.*

Fig. 47*C*. Six to 10 days after operation. The new sub-epithelial exudate only now becomes rapidly converted into new connective tissue (*not* granulations). New capillaries invade this zone from the stratum papillaris at the wound edges and remaining around the glands in the donor site. The epithelium now thickens considerably and develops numerous thick 'pseudo-pegs' which 'invade' the underlying new connective tissue, *simulating* regeneration of rete pegs.

Fig. 47*D*. At the fifteenth to twentieth post-operative day the new sub-epithelial connective tissue is fibrosing and apparently reacting to the 'invasive' epithelial pseudo-pegs. Consequently these pseudo-pegs are 'amputated' from their parent surface epithelium and undergo changes into internally keratinizing epithelial pearls and evoke foreign body reactions. The lower surface of the epithelium now becomes straight and scar-like. Collagen fibres are now present in greater quantity and the vascularity of the new connective tissue is reduced.

Fig. 47*E*. Forty days to years post-operatively—the epithelium does *not* regain the appearances of normal epidermis and is still devoid of 'true' rete pegs. The stratum papillaris of the dermis is *not* reformed and morphology of the fibrosed new connective tissue is different from the normal dermis, while elastic fibres do not reappear, at least for years. Some residual epithelial pearls, with associated foreign body reactions, may still be detectable.

opinions, we have found that the epithelium usually grows between the clot above and the denuded stratum reticularis of the dermis below. However, in some specimens where a considerable amount of plasma or serum had been expressed from, and deep to, the overlying clot, the epithelium frequently grew along *two* lines, firstly along the lower surface of the hardened clot, and secondly along the *upper* surface of the denuded dermis. The first line of growth usually ceases rapidly, while the second line of growth in contact with the denuded dermis proceeds actively to full coverage of the excised area. Only *after* the regenerating epithelium has covered the denuded surface does there appear, *below* this new epithelium, an exudate which seems to play an important part in the subsequent growth of the new connective tissue. As indicated, this sub-epithelial exudate is, at the outset, devoid of any blood cells; only subsequently do round cells emigrate into this sub-epithelial fluid matrix from the remaining portions of the stratum papillaris of the dermis about the hair follicles and other skin appendages, from the stratum papillaris remaining at the edge of the wound, from the perithelium and the perivascular cuffing. It should be stressed that new connective tissue could first be recognized in the healing wound at about the fifth to the seventh day, usually the latter. Before this the epithelium lay directly in contact with the stratum reticularis of the dermis or upon a sub-epithelial blister-like exudate.

These findings clearly establish that connective tissue regeneration occurs a considerable time *after* the prior and already well-advanced regeneration of the epithelium. Moreover, this connective tissue regeneration seems, in many respects, to be dependent upon the preceding activity of the regenerating epithelium. Although epithelization of the denuded surface is completed by the third to sixth day, growth of connective tissue has only *started* at about this time. We wish to emphasize that, *in the type of wound here studied, epithelialization is usually completed before there is any evidence of connective tissue regeneration.*

However, *epidermalization,* i.e. reorganization of the new *epithelium* into an *epidermal*-like structure, seems to be dependent, in turn, upon the development and maturation of the new connective tissue which begins to form beneath the new epithelium 5-7 days after the operation and 2-3 days *after* epithelization (i.e. covering with new epithelial cells) is completed. Changes in the new connective tissue, which forms in the bared area, are still in progress 8 years after the initial injury. Consequently the moulding of the new covering epithelium also continues for this length of time. Thus, while our evidence seems to establish that the *first* tissue to regenerate, during the healing of a Thiersch graft donor site, is the epithelium, the information available from this study also demonstrates conclusively that the subsequent maturation of the newly-regenerated sub-epithelial connective tissue exerts a profound effect on the later development of the epithelium itself. Initially the epithelium is thin, consisting of only 2-3 layers. However, after the new connective tissue appears in the sub-epithelial exudate, the epithelium itself thickens rapidly and considerably, and even gives rise to numerous thick invaginations into the underlying connective tissues. These thick, wedge-like invaginations by the rapidly thickening epithelium have given other investigators the impression that new rete pegs are being formed between the ninth and twelfth post-operative days. This impression is erroneous, as is shown by the fate of these epithelial invasions into the underlying connective tissue disclosed above. With the maturation of the newly-regenerated connective tissue, the early 'pseudo-pegs' of epithelium are ultimately eliminated and the epithelial-connective junction is altered from a rather serrated to a virtually straight line.

Our *findings concerning the epithelial connective tissue relations may be summarized as follows* (Fig. 47): The epithelium rapidly grows over and covers the denuded dermis with a thin layer of squames. Four to six days after the initial growth of the epithelium a sub-epithelial exudate appears into which round cells emigrate, and these subsequently give rise to new connective tissue from the fifth day onwards. The epithelium rapidly thickens thereafter, between the seventh and tenth post-operative days, and also becomes markedly 'invasive'. This invasive capacity of the epithelium evokes a marked connective tissue reaction, as to a foreign body, with consequent well marked round cell infiltration, giant cell formation and ultimate fibrosis. The fibrous tissue reaction to the epithelium which invades the new connective tissue during the healing of a donor site not only checks the epithelial growth but also plays a part in moulding the new *epithelium* into a structure which *resembles epidermis,* rather than a squamous stratified epithelium such as is normally found

lining the buccal cavity, oesophagus and vagina.

The 'pseudo-pegs' described by us as appearing in healing donor sites between the eighth and twentieth days seem to correspond to those epithelial downgrowths described by Brown and McDowell, Converse and Robb-Smith and Bishop as 'reconstituted *rete pegs*'. That these epithelial invaginations are *not* true rete pegs at all, but temporary epithelial downgrowths into the new connective tissue, is borne out by our study of the fate of these invasions as well as of the morphology of the 'epidermis' which ultimately covers the donor site 2-8 years after the graft was taken. 'True' rete pegs are not detectable in healed Thiersch donor sites even 8 years after the graft was removed. This type of healing, while not comparable with that seen in 'true' scars associated with contracture, cannot be regarded as complete reconstitution of the skin. We have referred to the healing which supervenes in Thiersch donor sites as 'healing without scar formation'.

Since our findings, acquired during the past 5 years, contradict the time-honoured, though apparently erroneous opinions about the healing of excised wounds, we were highly gratified to encounter strong confirmatory evidence in a recent publication by Clark and Clark. These authors have shown regenerating epithelium to have marked fibrinolytic effects on the medium into which it grows. They have also demonstrated, most elegantly, that *regenerating epithelium exerts profound inhibitory and modifying effects on the growth of new blood vessels as well as upon the type and organization of fibres deposited in the immediate vicinity of the epithelium* which grows into the observation chambers studied by them. In view of the excellence and relevance of the findings of Clark and Clark, we quote them *in extenso*. They state:

'... the fibrin net persisted for a short time, after which it was replaced by a new ingrowth of connective tissue cells that followed the contour of the epidermal border. This was, in turn, succeeded by the formation of dense connective tissue fibres that differed in pattern and appearance from the connective tissue that developed generally in the chambers . . . The chief difference noted in the fibres that developed near the epidermis . . . as compared with the others, were their regular arrangement as fibres parallel with the line of epidermal, rather than parallel with the line of vascular advance; their density, and their larger cross-section size. They seemed to develop more rapidly than the general fibrous network, and also, at times, to develop immediately next the epidermis before the blood vessels had arrived. This modification of connective tissue fibre pattern in the cases in which epidermis invaded and differentiated in rabbit's ear chambers have also been observed by Sterns (1940).'

In describing the *effects of epidermis on the growth of blood vessels,* Clark and Clark remark:

'... the presence of epidermis in the table space had a marked effect upon the growth and pattern of the blood vessels . . . In addition to its action in blocking the advance of new blood vessels and in forcing changes in newly-formed vascular networks, the presence of epidermis also influences the *direction* of blood vessel growth.'

Our findings on the final growth of epithelium across Thiersch donor sites, the subsequent development of a sub-epithelial fluid exudate (which seems to provide an ideal medium for connective tissue regeneration) as well as our observations on the ultimate relations between the subsequent development and maturation both of epithelium and of connective tissue, are in complete conformity with Clark and Clark's descriptions. As indicated before, during the healing of *incised* wounds epithelium is also the first tissue to regenerate and new connective tissue appears in the transected dermis only some days after epithelization of the incision is complete. In this previous study we also described the apparent organizing effects of new epithelium on the growth of connective tissue and upon the direction in which new fibres were deposited.[28-30] We also found, several years ago,[27] that regenerating epithelium promotes the rapid deposition of fibres (reticulin and collagen) in previously almost fibre-free granulations that were treated by grafting. This effect of epithelium supervenes within a few days of contact with the granulations is evidenced, initially, by an increase in reticulin fibres about the primitive blood vessels in the granulations and, soon thereafter, by the appearance and rapid increase in collagen fibres which were invariably laid down parallel to the epithelial-lined surface. It may be that the grafting of extensive injuries, involving tissue loss, prevents serious scar formation and contracture by virtue of the extent, nature and direction of fibre deposition within granulations.[27] On the other hand, rapid healing of wounds, e.g. of second degree burns or Thiersch donor sites, in which scar formation is absent, may be dependent not only upon the ability of new epithelium to promote fibre deposition, but also upon the apparent effect of the *early* presence of epithelium on the course of the healing process generally and particularly on the rate of development and on the organization of the first phases of connective tissue growth.

We have also demonstrated in our previous study that in the healing of Thiersch graft donor sites, as in the healing of incised wounds, the new connective tissue which is deposited is *not* morphologically identical with what is described in most textbooks as 'granulation tissue'. We have provided reasons for this view that a distinction should be drawn between granulation tissue, as generally understood, and what we have described as 'new connective tissue' appearing during the healing of incised wounds and also during the repair of Thiersch graft donor sites.

We found (in a previous study, as in the present one) that the maturation of regenerated connective tissue in turn influences and even seems to restrict the 'invasive' behaviour of new epithelium. The formation and subsequent maturation of new connective tissue which develops during the healing of Thiersch graft donor sites also seems to play a part in determining the ultimate morphology of the epithelium which covers the cutaneous defect.

4. Possible Implications of the Above Findings for Understanding the Pathogenesis of Skin Cancers

In several studies [27-30] we have reviewed much of the literature dealing with the possible role of the connective tissues in the pathogenesis of skin cancers occurring spontaneously in Man and produced by the application of various carcinogens to the skin of experimental animals. Orr [72] and Linell [51] and many others before them (see these authors for bibliographies) have drawn attention to the marked changes in the dermis consistently supervening in association with methylcholanthrene-induced experimental skin carcinomas, while Vernoni [93] has stressed the possible importance of similar dermal alterations in the pathogenesis of human skin carcinomas.

More recently Jolles [45, 46] has reviewed the literature dealing with the possible role of connective tissues in the histogenesis of carcinomata in man. He hypothesized that the growth of carcinomas might be determined by the reactions in the surrounding connective tissues. On the basis of his theory, Jolles devised a new and an apparently highly successful method of 'grid' X-ray therapy of carcinoma in which rapid and very satisfactory resolution of large carcinomas could be attained on relatively very small doses of irradiation. Jolles aimed his therapy *not* at the epithelial component, but at the connective tissue which, he claimed, is induced to form a diffusible anti-carcinogenic substance, which was ultimately responsible for inhibiting the growth of the treated carcinoma.

In the first published report of the activities of our group, we showed that it is possible to influence profoundly the regeneration of epithelium in Thiersch graft donor sites by applying grafts of trypsin-treated homodermis;[27] so much so, that at 17 days after the application of such homodermal grafts to freshly prepared donor sites, the epithelial regeneration was so active as to simulate closely local neoplasia of epidermis. This markedly hyperplastic epithelium in turn seemed to evoke a marked connective tissue response which apparently ultimately inhibited the epithelial growth. This pattern of reactions was later confirmed by being elicited consistently in 11 human volunteers.[30] At that time we suggested that these findings might indicate that the state of the dermis played a role in the pathogenesis of neoplasia. It was also hypothesized that alterations in the chemical constitution of the dermis, arising as a result of *endogenous* metabolic disorders, or induced locally by extraneous stimuli, might be of considerable significance in determining whether a localized epidermal hyperplasia or regeneration would subside or would progress ultimately to become neoplastic. In the present study we have shown that the epithelium in a healing donor site seems to stimulate the regeneration of connective tissue and that once connective tissue has appeared beneath the epithelium, the latter becomes hyperplastic and invasive. Subsequent maturation of the connective tissue seems to inhibit the further growth of epithelium and, in fact, destroys the invasive 'pseudo-pegs'.

Our studies, reviewed in the light of the above data, and particularly in the light of the information recently provided by Clark and Clark as well as by our own more direct studies,[31a] adduce further and perhaps more direct evidence that the role of connective tissue in the pathogenesis of carcinomas deserves far more intensive attention than it has hitherto received.

5. Some Possible Practical Applications of the Foregoing Findings

Among the possible practical applications are the following:

(a) *Time for Removal of Dressings from Graft Donor Sites and Second Degree Burns.*

We have shown that at certain stages of healing the epithelium is very thin and virtually unattached to the bared area, except by virtue of the connexions with its parent epithelium lining the sweat and sebaceous gland ducts and the hair follicles. This is especially so between the fourth and seventh days, when a sub-epidermal 'blister-like' new exudate appears. If the dressing is removed at this time, and the surface clot is disturbed or ruptured, or sticks to the dressings and is torn off, then the very thin and loosely attached epidermis will almost unavoidably be interfered with. Only after the tenth day, when new sub-epidermal connective tissue regeneration is well advanced, and when the epithelium over the donor site has thickened markedly and has even sent numerous 'pseudo-pegs' into the underlying connective tissue, can the epithelium be considered to be sturdy and reasonably well attached. At this time, too, since keratinization of the surface epithelium is already well under way, a natural plane of cleavage is provided, like a perforated line in a copy book, for the easy seperation of the clot from the underlying epithelium. Our experience in experimental animals has substantiated these suggestions as highly practicable.

(b) *Time for Harvesting a Second Crop of Epithelium from a Previously Used Donor Site.* In their excellent monograph on skin grafting Brown and McDowell mention that, when this procedure is indicated, they usually remove a second layer of tissue for grafting on the nineteenth post-operative day. We have shown that the epithelium is at its maximum thickness between the fourteenth and sixteenth post-operative days and that thereafter it becomes progressively thinner and also gradually loses its invasive 'pseudo-pegs' during the next 10-20 days. On our findings the best time to harvest a second crop of epidermis would be about the fifteenth to sixteenth day after the first thin split skin graft was taken, when the epithelium is thickest. This would seem reasonable if the objective is to obtain a thick layer of actively growing epithelium together with a suitable associated lamina of young, vigorously growing and highly cellular, relatively fibre-free connective tissue. Admittedly, at this time (about the fifteenth to sixteenth day) bleeding would probably be more profuse than at, say, the twentieth day, when the new connective tissue is rapidly becoming collagenized with consequent diminution of blood vessels. Removal of epidermis plus new connective tissue, at the suggested time, would also yield less highly differentiated structures which may be cosmetically more valuable.

(c) *Assessment of Results of Skin 'Planing'.* The method of 'dermabrasion' for superficial skin defects and acne scars is equivalent to the removal of very thin split-skin grafts. In the light of our findings it would seem unwise to assess the outcome of such an operation for at least 2-3 years, more especially in view of the progressive fibrosis of the new connective tissue and the associated alterations in the overlying regenerated epithelium. Final assessments of 'dermabrasions' should be avoided for at least 2 years. The patient may anticipate a progressive improvement at least for this period of time, provided serious scarring is not to be anticipated in the light of the depth of the 'planing'.

(d) *Possible Use of Dermal Homografts in Treatment of Extensive Burns.* The rationale usually propounded for the use of skin grafts in the treatment of extensive deep skin injuries is that by providing a source of *epithelium* (much of which is lost by virtue of the injury), scarring and subsequent contracture will be prevented. Billingham and Reynolds clearly demonstrated that a free supply of viable epithelium *failed* to prevent wound contracture. They concluded that '. . . at least *some dermis* is necessary to do this'. We postulated that unless the connective tissue response in an injury was modulated by the influence of the abutting damaged dermal tissue and the epithelial new growth, then wound contracture and scarring would inevitably result. We have been able to modify, in a most significant manner, the course of healing and rate of contracture of deep excised wounds by the application of homodermal grafts in experimental animals. This work is still in progress and will be reported on in detail later.

SUMMARY AND CONCLUSIONS

1. The healing of thin split-skin (Thiersch) graft donor sites has been studied in a series of 12 healthy volunteers—7 European, 1 African, 3 Indian and 1 Mulatto. A total of 69 biopsies of the healing donor site together with the untraumatized neighbouring skin were taken during the first 80 post-operative days. In addition, 2 further biopsies were studied, one from a 2-year-old and one from an 8-year-old healed donor site. All studies were made on *serial* sections of all biopsies.

2. It was found that the first tissue to regenerate is the epidermis. The epithelial regeneration commences within 24 hours of

operation and arises from the wound edge, the hair follicles, sebaceous and sweat glands, in this order of activity.

3. Newly regenerated epithelium spreads across the *donor site in direct contact with the denuded stratum reticularis of the dermis,* initially unaccompanied by any connective tissue re-growth. The new epithelium seems to secrete some proteolytic enzyme which allows it to grow actively beneath the clot, apparently by lysing the fibrin fibres attaching the surface clot to the raw area.

4. The untraumatized epidermis adjoining the wound undergoes marked changes, with the formation of tall columnar epithelial cells occupying the basal 3-5 layers of the epithelium, and with the appearance of excessive numbers of prickle cells.

5. Four to six days post-operatively, when epithelization of the denuded surface seems to be completed, there appears, below the new epithelium, a cell-free protein- and glycogen-rich exudate. Round cells from the blood vessels and from recent perivascular infiltrations migrate into this initially cell-free sub-epithelial exudate. The new connective tissue, which starts developing in the donor site between the fifth and seventh post-operative days, seems to arise solely from these round cells and *not* from the mobilization of pre-existing contiguous dermal fibrocytes.

6. The denuded original stratum reticularis of the dermis remains remarkably inert throughout the healing process.

7. After the appearance, sub-epithelially, first, of a cell-free exudate and later of new connective tissue, the 2- or 3-layered new squamous epithelium increases in thickness rapidly, due to marked mitotic activity within the new epithelium itself and also due to changes in the morphology and arrangement of these cells.

8. The initial and subsequently regenerating epithelium is easily distinguishable, from the outset, by the accumulation within these epithelial cells of large quantities of stainable glycogen, and by the loss of the stratum granulosum. Parakeratosis therefore supervenes over the healing donor site until about the ninth post-operative day, when keratohyalin granules reappear in the upper layers of the regenerated epithelium associated with a loss of stainable glycogen.

9. The marked hyperplasia of the epidermis, associated with the appearance of new connective tissue at the fifth to seventh post-operative days, continues progressively until about the fourteenth to seventeenth days. During this period (fifth to about the fifteenth days), the regenerating epithelium also gives rise to numerous thick 'spurs' of cells which seem to invade the new sub-epithelial tissue. These 'spurs' have been described by previous authors as the reappearance of rete pegs. Since we have shown these 'spurs' to be subsequently resorbed as a result of the vigorous connective tissue reaction which they evoke, we have called them 'pseudo-pegs'. After the twentieth day the initially serrated lower surface of the regenerating epithelium becomes straight, due to the amputation and encapsulation, by connective tissue, of the 'pseudo-pegs' which thus give rise to numerous internally keratinizing epithelial 'pearls'.

10. The new epithelium seems to exert a profound effect in promoting the regeneration of new connective tissue. It may well be that the avoidance of scars, when some local epithelium remains in the wound or when epithelium is provided by the application of split-skin grafts to deeply excised wounds, may be attributable directly to the effects of the epithelium and associated dermis of the graft on the *nature* and the extent of the new connective tissue *initially* formed in the wound, as well as on the subsequent *maturation* of this new connective tissue. The latter, in healing donor sites, is *not* morphologically comparable with 'true' granulation tissue. The original epithelial covering of the donor site is modified or remoulded by the maturation of the new connective tissue. This remoulding of the epithelium into an epidermal-like structure continues for at least 2 years post-operatively and ultimately results in the formation of a scar-like epidermis.

11. Whereas we have shown that the epithelial regeneration initially exerts a profound effect in stimulating the growth of connective tissue in the wound site, we also found that the subsequent development of the connective tissue in turn significantly influences the activity, invasive properties and subsequent remoulding of the epithelium.

12. The epithelial-connective junction normally existing in skin undergoes marked changes, both at the wound edge as well as over the wound site. The development of a normal-appearing epithelial-connective junction or epidermal-dermal junction, is not attained for at least 2 years after the Thiersch graft is taken. New elastic fibres are first seen in the healed donor site only 2-8 years after the injury. The original fine sub-epithelial fibre net-work, characteristic of the normal stratum papillaris of the dermis, does not reappear for

8 years, and probably never reappears. The vascular net-work in the newly formed connective tissue in the donor site, also probably fails to re-acquire its normal morphology.

13. The original collagen in the dermis contiguous with the wound undergoes a change in morphology and in staining reaction, to resemble first 'fibrinoid' and subsequently, what we have called 'elastotic degeneration'.

14. Some of the possible practical implications of our findings are discussed, and attention is directed particularly to:

(a) The importance of more intensive histological, histochemical and chemical study of epithelial-connective tissue relations in the pathogenesis of carcinoma.

(b) The fact that, since no new connective tissue is formed until about the fifth to seventh post-operative days, the epithelium covering the wound is very loosely attached until about the eighth day. It is therefore probably unwise to remove dressings until about the tenth post-operative day, in order to avoid damaging or accidently removing the new epithelium.

(c) Since the epithelium which regenerates over the donor site undergoes marked hyperplasia between the fifth and fifteenth post-operative days, and is probably at its thickest between the fourteenth and seventeenth days (and thereafter becomes considerably thinner) if the harvesting of second crops of grafts from the same donor site are indicated, these should probably be taken between the fourteenth and seventeenth post-operative days. It is during this latter period that the epithelium is thickest and that the connective tissue is growing most actively.

(d) The possibilities of a rationale for using *dermal* homografts in the treatment of extensive, deep skin wounds are briefly considered.

15. Finally, our findings clearly indicate that the traditionally accepted descriptions of the healing of wounds involving loss of skin substance (which maintained that the epithelial regeneration is dependent on *preceding* connective tissue growth first filling the gap) can no longer be regarded as acceptable in their entirety.

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